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TITLE: DNA Copy Number Signature to Predict Recurrence in Early-Stage Ovarian Cancer

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| 13. SUPPLEMENTARY NOTES | | | | | |
| 14. ABSTRACT : The second year of this award included the following tasks Major Task 2: To determine the copy number gain and loss for early stage high grade ovarian cancers through IlluminaHumanOmniExpress-FFPE BeadChip system <ul style="list-style-type: none"> Subtask 1 DNA sample quality assessment and processing (FFPE restoration) to prepare chip compatible samples. Months: 9 - 18 Subtask 2 Genomic abnormality analysis. Months 9 - 18 Milestone(s) Achieved: <i>Obtained DNA samples from 384 recurrent and non-recurrent early-stage ovarian cancers for the proposed copy number study.</i> All samples have been analyzed for 5X Illumina NextGen sequencing to detect DNA-CNV. Major Task 2: Integrated analysis of the copy number variation result and the RNAseq results obtained from a parallel DOD study of the PI (OC110628). This task is still ongoing we will accomplish by end of this no cost extension year, 7/31/2017. | | | | | |
| 15. SUBJECT TERMS Early Stage Ovarian Cancer, genomic predictive signature, recurrence | | | | | |
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Introduction

The survival of women with high-grade epithelial ovarian cancer is directly related to the spread of the tumor. Women with disease limited to the pelvis do well with many being cured, while those patients whose tumor has spread outside of the pelvis suffer recurrences and the majority will die from the disease. Nevertheless, the standard of care for patients with high-grade ovarian cancer is surgery followed by 6 cycles of chemotherapy (carboplatin/taxol) regardless of the spread of the tumor. Although some early stage patients are benefiting from this strategy, approximately 50-60% of patients with high-grade early stage cancer will not develop recurrent disease even in the absence of chemotherapy. These patients thereby suffer unnecessary short and long-term toxicities of chemotherapy with no benefit. Thus, the development of accurate biomarkers predictive of tumor recurrence becomes essential to identify women with early-stage disease who will benefit from chemotherapy while sparing the rest the unnecessary treatment with quality-of-life and cost-effectiveness ramifications. This approach parallels efforts in breast cancer where tests like “oncotype dx” provide valuable information on disease recurrence to women with early stage breast cancer. To the best of our knowledge, there is no available large-scale molecular characterization of early-stage ovarian tumors. Here, we propose to develop genomic signatures correlated with clinical outcomes and in particular tumor recurrence for early stage ovarian cancer. The status of DNA copy number variation (CNV) in recurrent and non-recurrent early stage high grade ovarian cancer will be investigated using a large, fully annotated, consortium cohort of 1628 samples from clinical trials. Integrated analysis will be performed by combining the gene expression profiles obtained from a recently terminated ongoing DOD project (W81XWH-12-1-0521) using the same 1628 samples with the gene copy results from this proposal. Through the integrated analysis of deleted, amplified and aberrantly expressed genes in early stage ovarian cancer, we expect to develop predictive biomarkers for future prospective stratification of women with early stage ovarian cancer to adjuvant carboplatin/taxol chemotherapy versus careful follow-up. This study will also contribute the identification of therapeutic biomarkers and stratification of early stage ovarian cancer patients most likely to benefit from targeted interventions.

KEYWORDS: Early Stage Ovarian Cancer, genomic predictive signature, recurrence, DNA copy number variation

Research Accomplishments

Major Task 1: Obtain DNA samples from consortium specimens. Months 3-8

The tasks for the first project period included: 1) Obtain DNA FFPE specimens collected through the consortium of early stage high grade ovarian cancer, 2) Analyze about 50% of all required samples through IlluminaHumanOmniExpress-FFPE BeadChip system.

Through a previous DOD award (W81XWH-12-1-0521) we have: 1) Established an international consortium through which we have collected 1628 FFPE samples of serous ovarian cancer, 2) identified 592 early-stage high-grade ovarian cancers with 5-year follow-up, clinical annotation and accurate pathological review (228 recurrent and 364 non-recurrent), 3) established a specimen repository and clinical data inventory at MGH, 4) Sequenced RNA from these tumor samples, 5) Obtained preliminary RNAseq data indicating the need to analyze 384 samples at a

ratio of 2 non recurrent tumors versus 1 recurrent tumor to obtain a statistically significant genomic signature.

In addition, in the last budget period of this award we have optimized an SOP for double DNA and RNA extraction from our FFPE tissues and a procedure to randomize these samples to avoid any batch effect.

Major Task 2: To determine the copy number variation (CNV) for early stage high grade ovarian cancers through Illumina HumanOmniExpress-FFPE BeadChip system. Months 9-18

In the first year of funding we had also selected the RPCI Genomics Shared Resources at Roswell Park Cancer Institute as a core facility for CNV analysis of our samples, but unfortunately, the Illumina Hybridization Chips utilized for this analysis have revealed to be inefficient as we had less than 30% hybridization in more than 50% of samples of the initial batch of 48 samples that was analyzed. This was an unfortunate result that delayed the final analysis of all samples and identification of a DNA signature that predicts for recurrence in early stage high grade ovarian cancer.

We have thus submitted this initial batch of 48 samples to the DFCI Molecular Biology Core Facilities to test a DNA CNV analysis procedure based on shallow (5X) whole genome sequencing (WGS) technology using the Illumina NextGen platform. Shallow WGS has recently emerged as an alternative to microarray for copy-number detection, including the utilization of FFPE DNA as input. Through working with the DFCI core, we have overcome two major obstacles to ensure that copy-number can be reliably estimated through standardized workflow of segmentation. This include: 1) elimination of the amplification bias during the library preparation by using a PCR-free protocol, and 2) elimination of the necessity of using a reference 'normal' specimen by using observed-sequencing-depth across the genome to infer copy-number variation. A bioinformatic pipeline requiring the correction of sequencing bias has been executed using *QDNAseq* R/Bioconductor package, which implements the correction of low mappability, extreme GC content, as well as difficult regions known to be associated with Illumina platform as previously disclosed by large studies like ENCODE or 1000 Genome Project. The results of this shallow WGS sequencing procedure in the first 48 samples are attached to this progress report and were deemed viable for the identification of a signature for recurrence.

Overall, given the technical difficulties we had in processing our FFPE tumor tissues for both RNAseq under the DOD award W81XWH-12-1-0521 and DNA-CNV under current award, we have requested a one year no cost extension of this current award to terminate all data analysis and integration.

Major Task 3: Integrated analysis of the DNA-CNV results and the RNAseq results obtained from a paralleled DOD study of the PI W81XWH-12-1-0521. Months 18-24

All 384 early stage/high grade ovarian cancer FFPE samples have now been sequenced and divided in training and validation sets and data analysis is ongoing. It is to note, that throughout these years we have received an additional funding from the Ovarian Cancer Research Fund (OCRF) complementing these studies that includes analysis of micro-RNA expression in these samples. Thus, we started a parallel analysis of RNAseq, DNA-CNV, and miRNAseq. To avoid

any bias, we decided to analyze all these genomics data in parallel at the same time and then integrate the results. We predict to have conclusive data by the end of 2017.

Results disseminated to communities of interest: We have created a news letter that is being distributed every 2 months to communities of interest (attached). This news letter updates the communities on the status of the project and keeps them engaged. It may be used to ask for more material. Please find attached the first version of the letter that was submitted when this project started.

Actual or anticipated problems or delays and actions or plans to resolve them: None

IMPACT

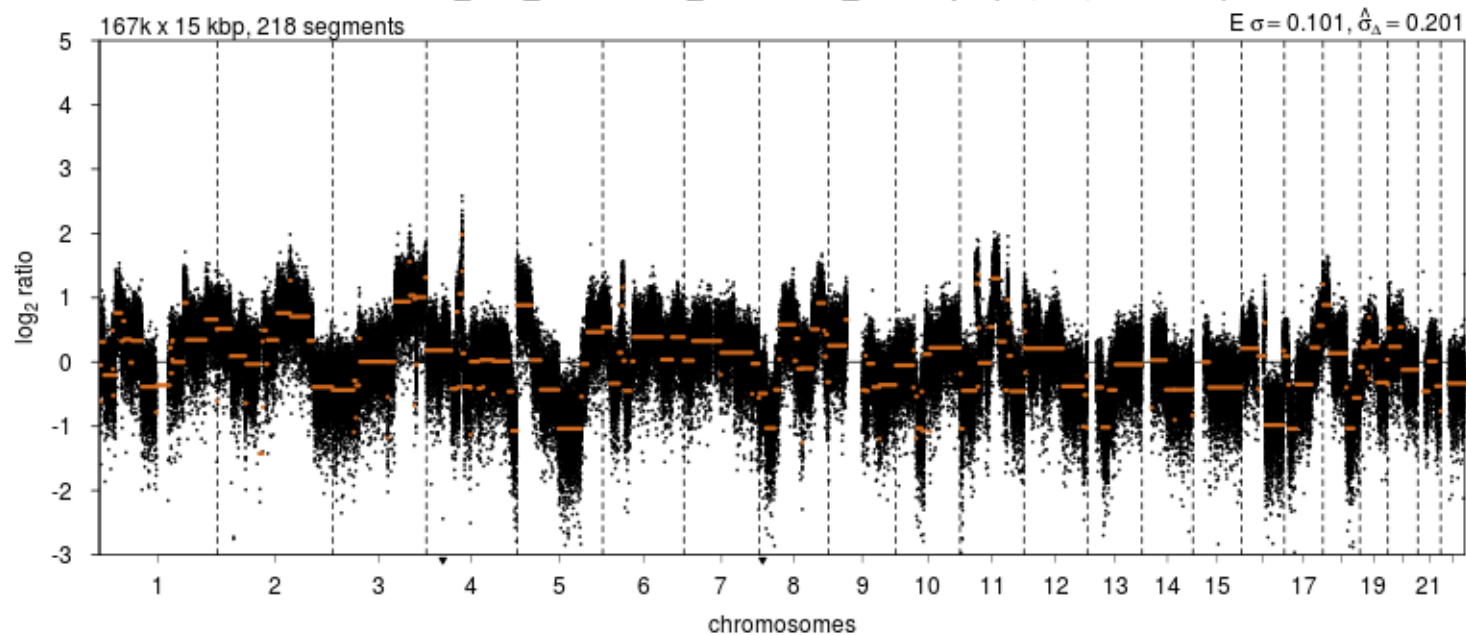
Impact on the development of the principal discipline(s) of the project: Creation of a well annotated biorepository of early-stage tumors allows performing correlative clinical and genomic studies on these tumors that are so poorly characterized and yet significantly affect the life of so many women.

Impact on other disciplines: Nothing to report

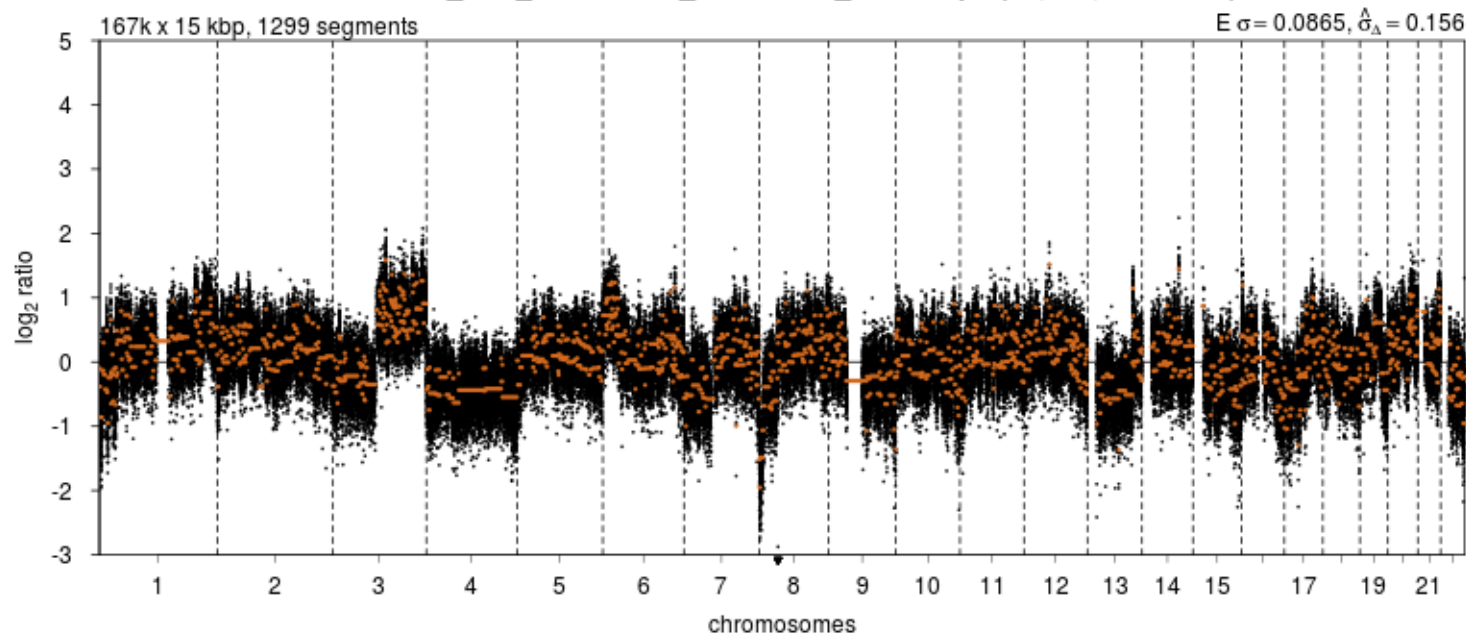
Impact on technology transfer: We anticipate that genomic discoveries in this project will have commercial application.

Impact on society beyond science and technology: Nothing to report

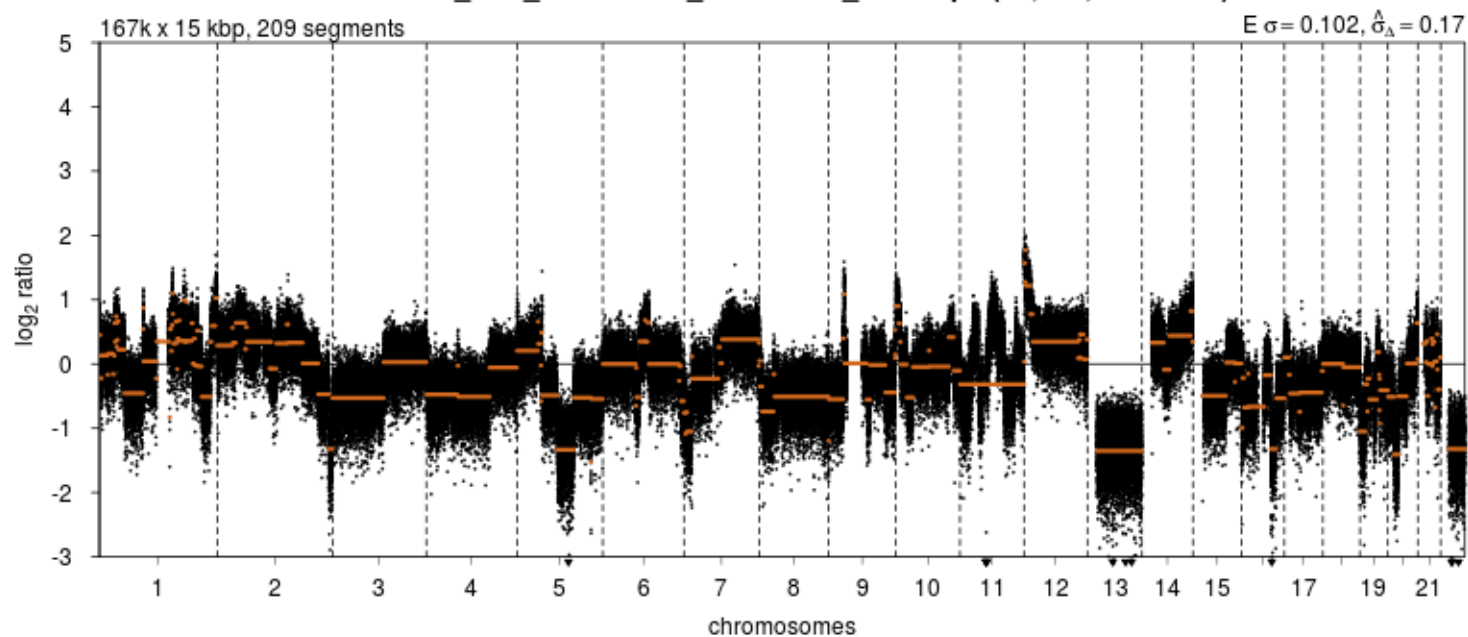
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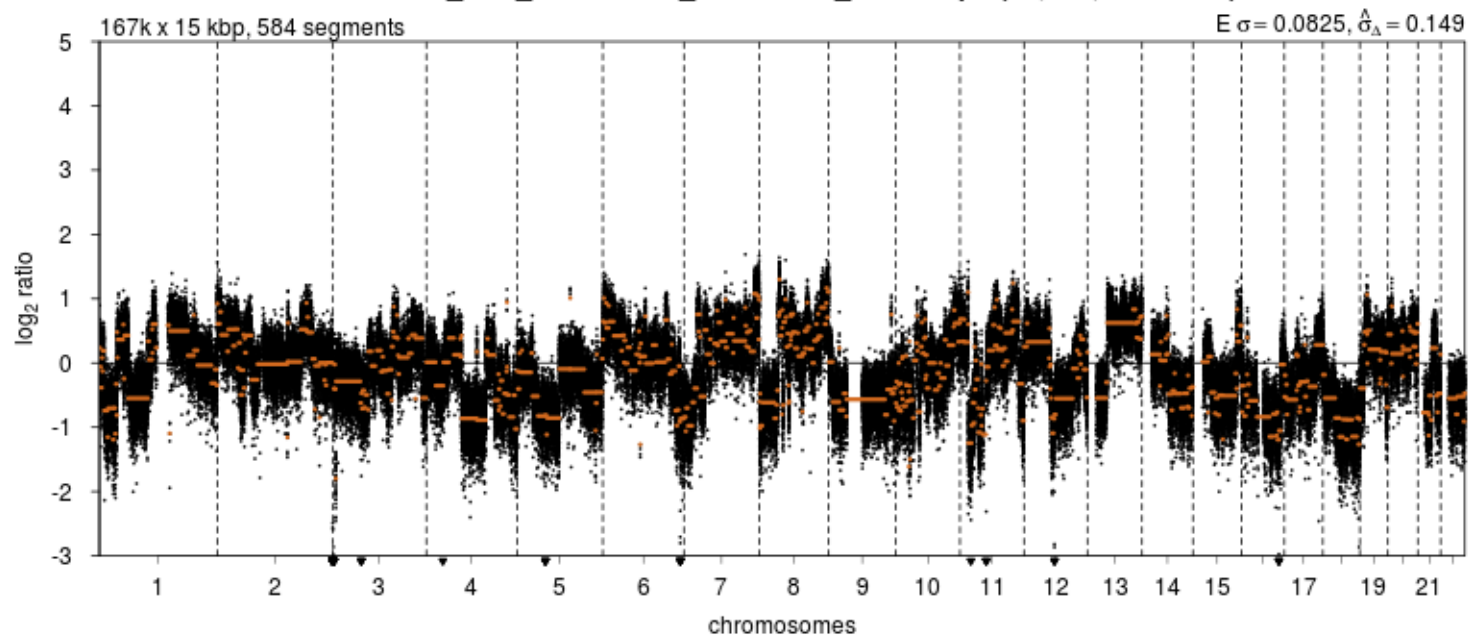
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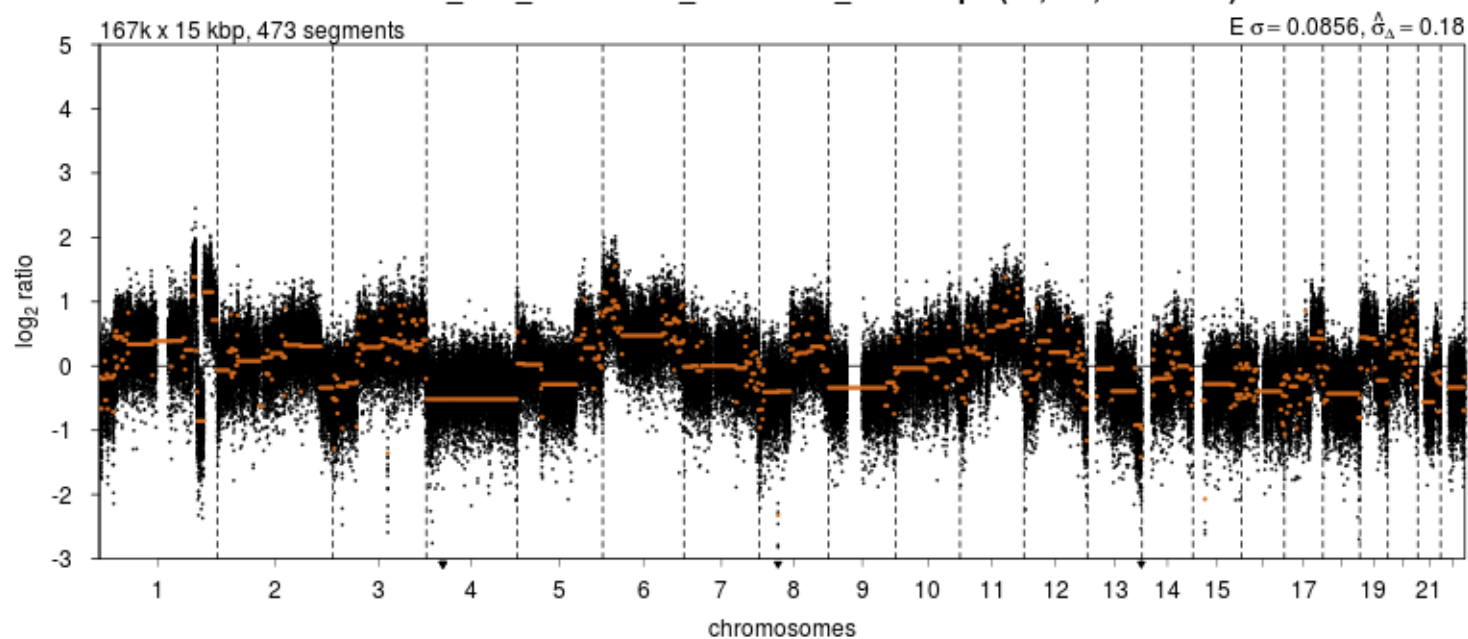
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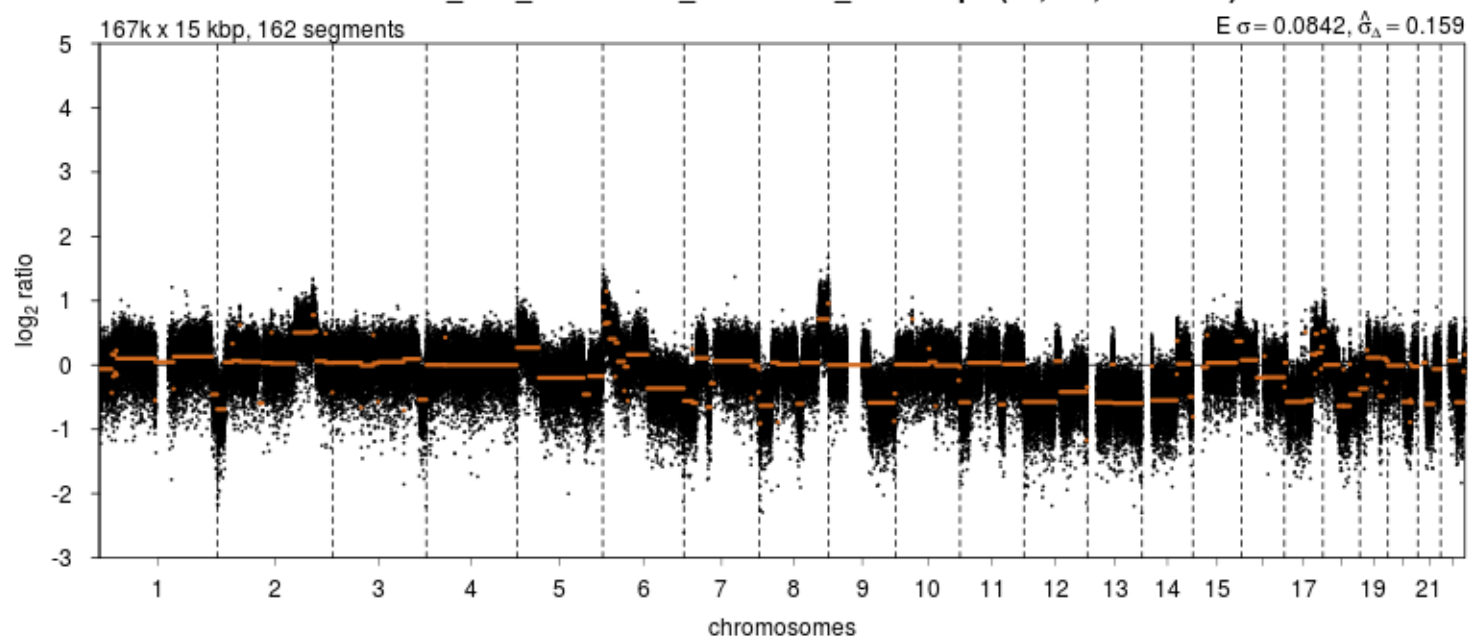
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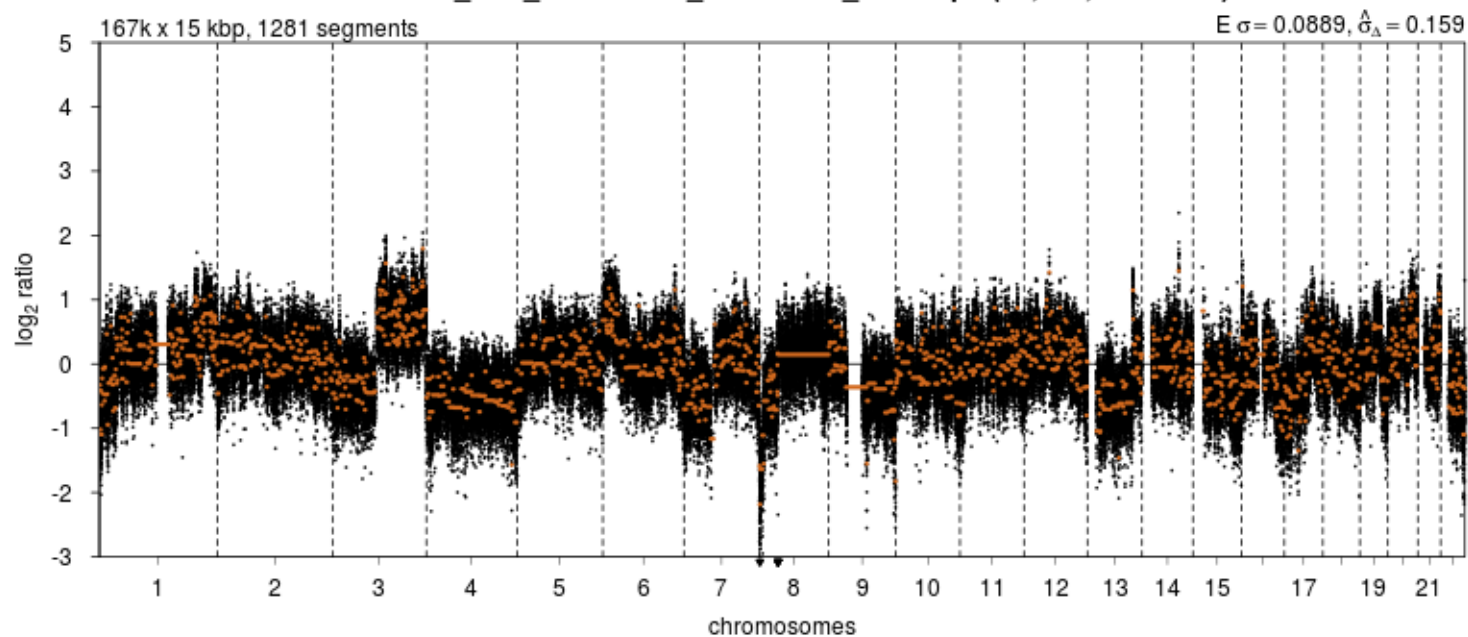
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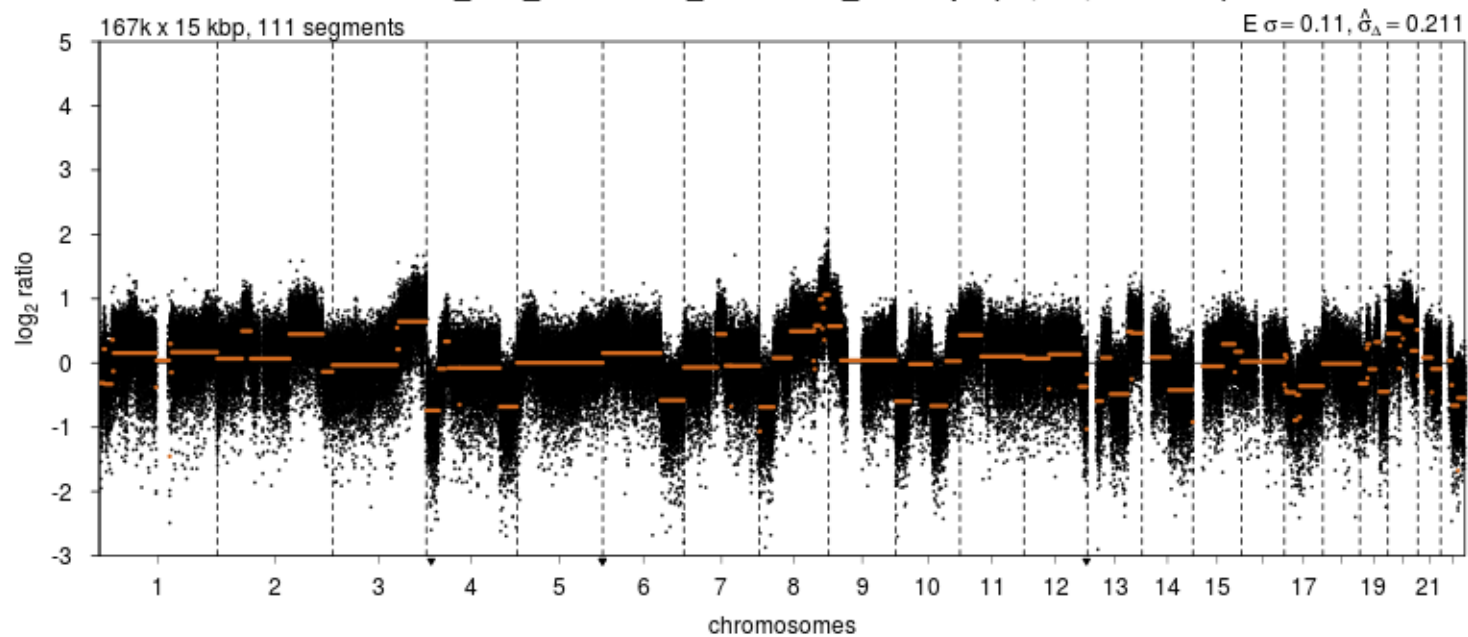
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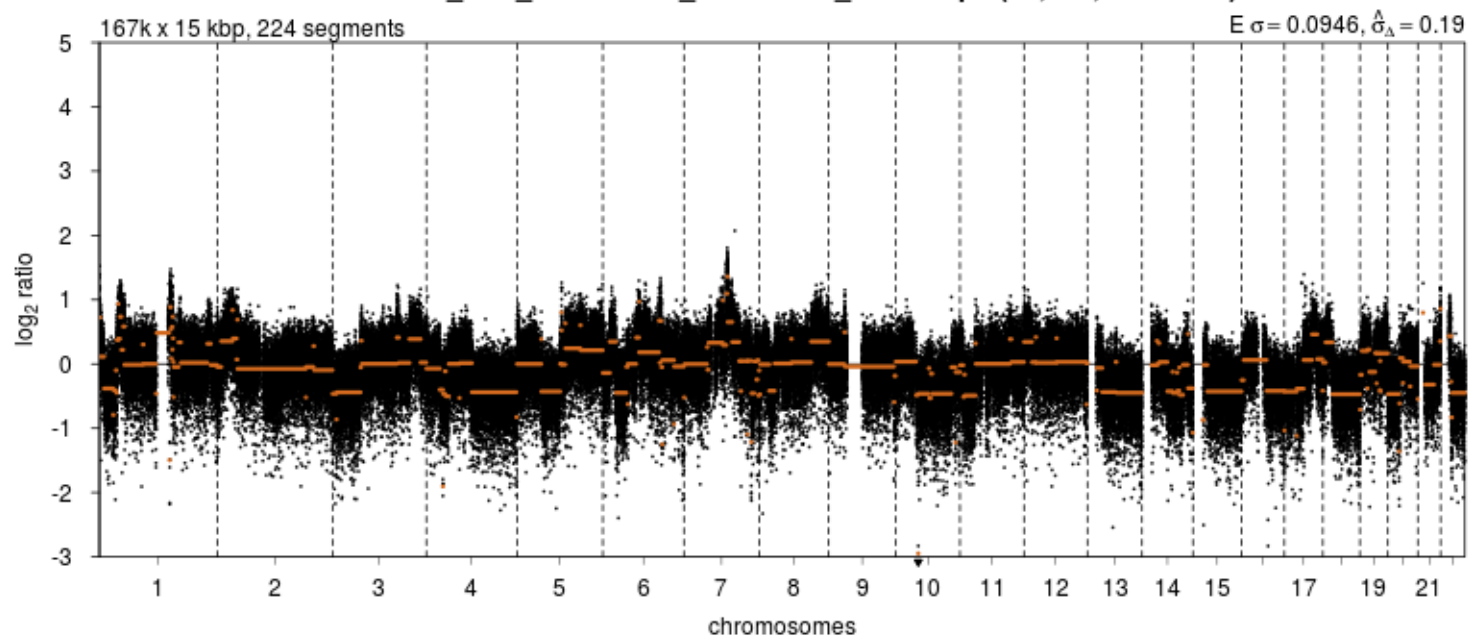
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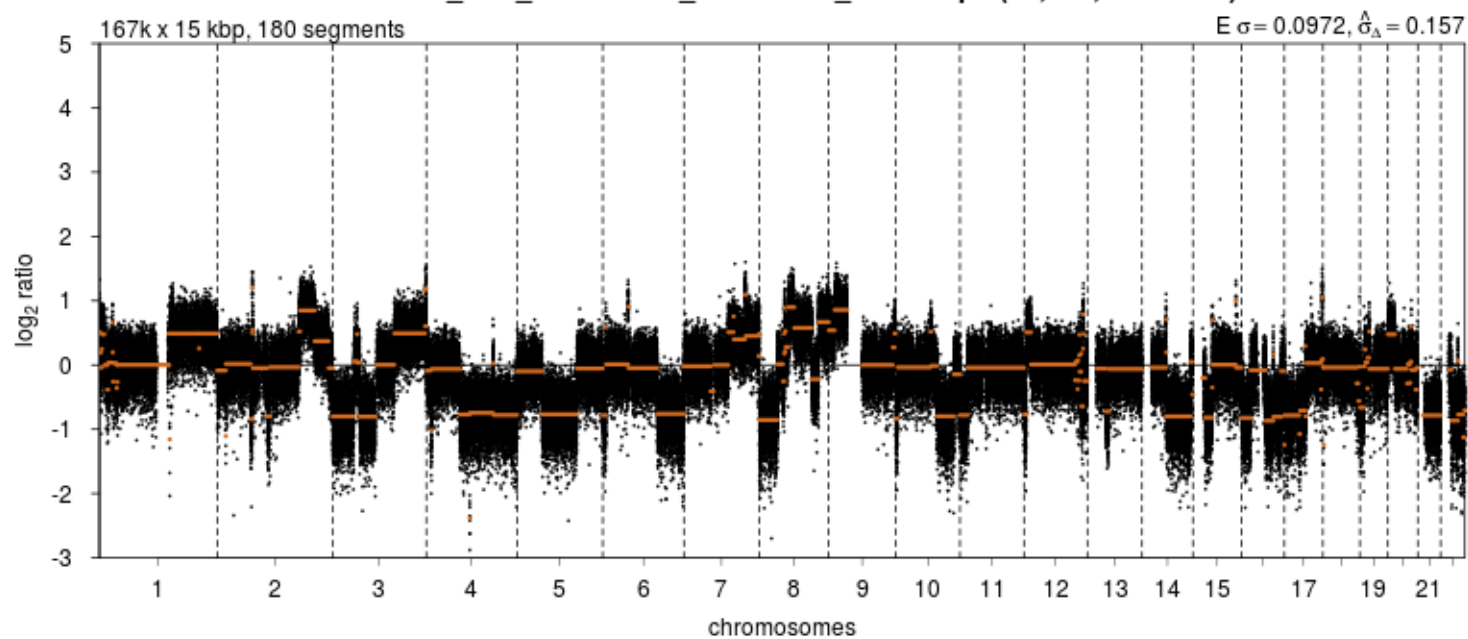
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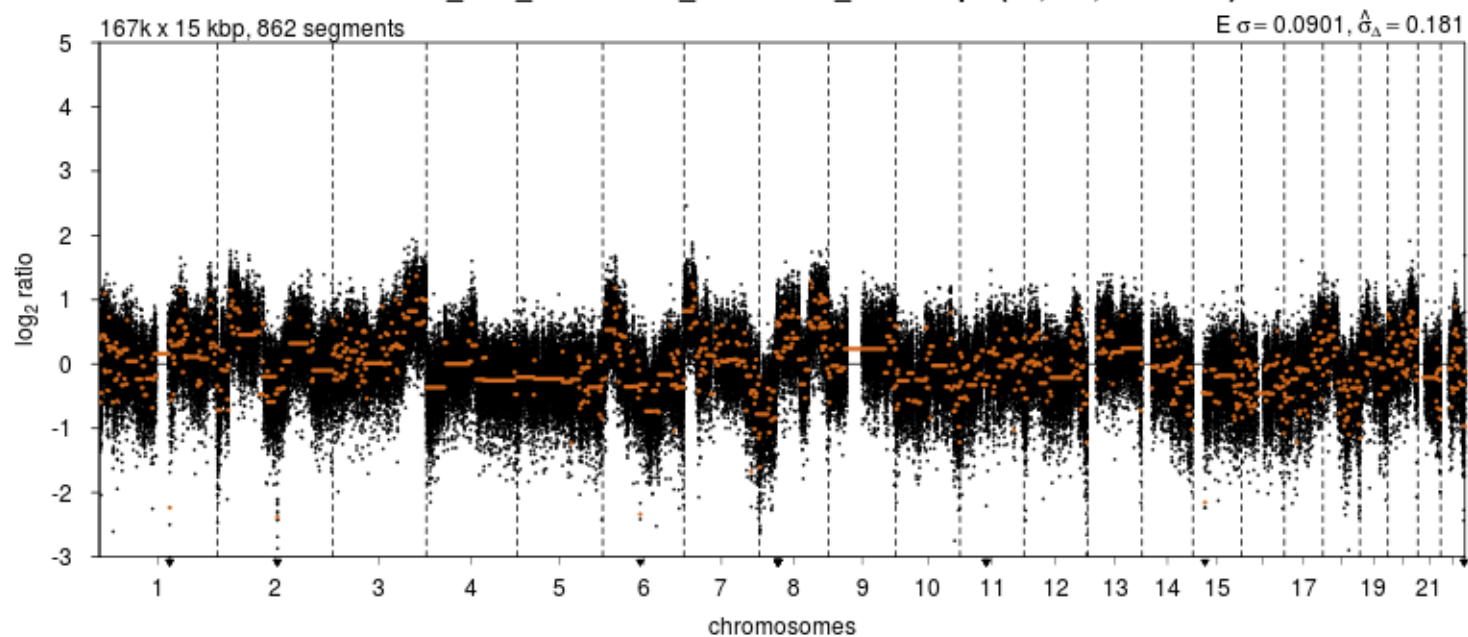
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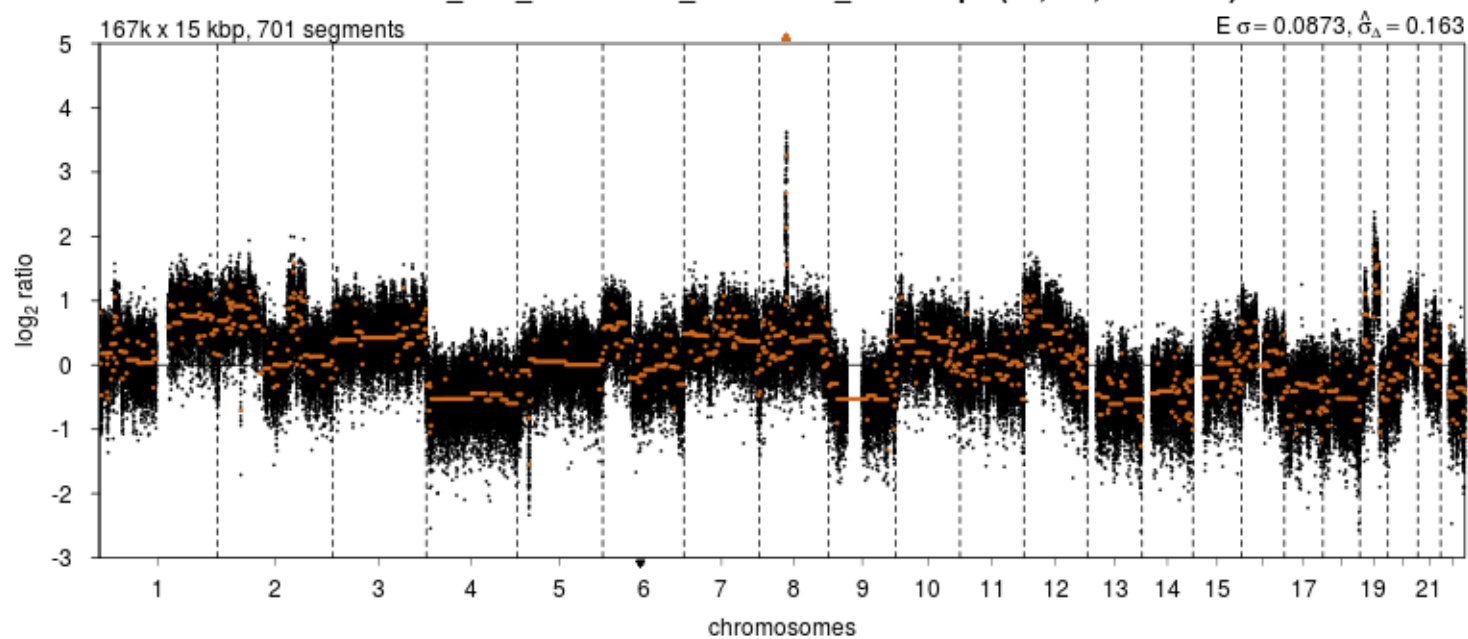
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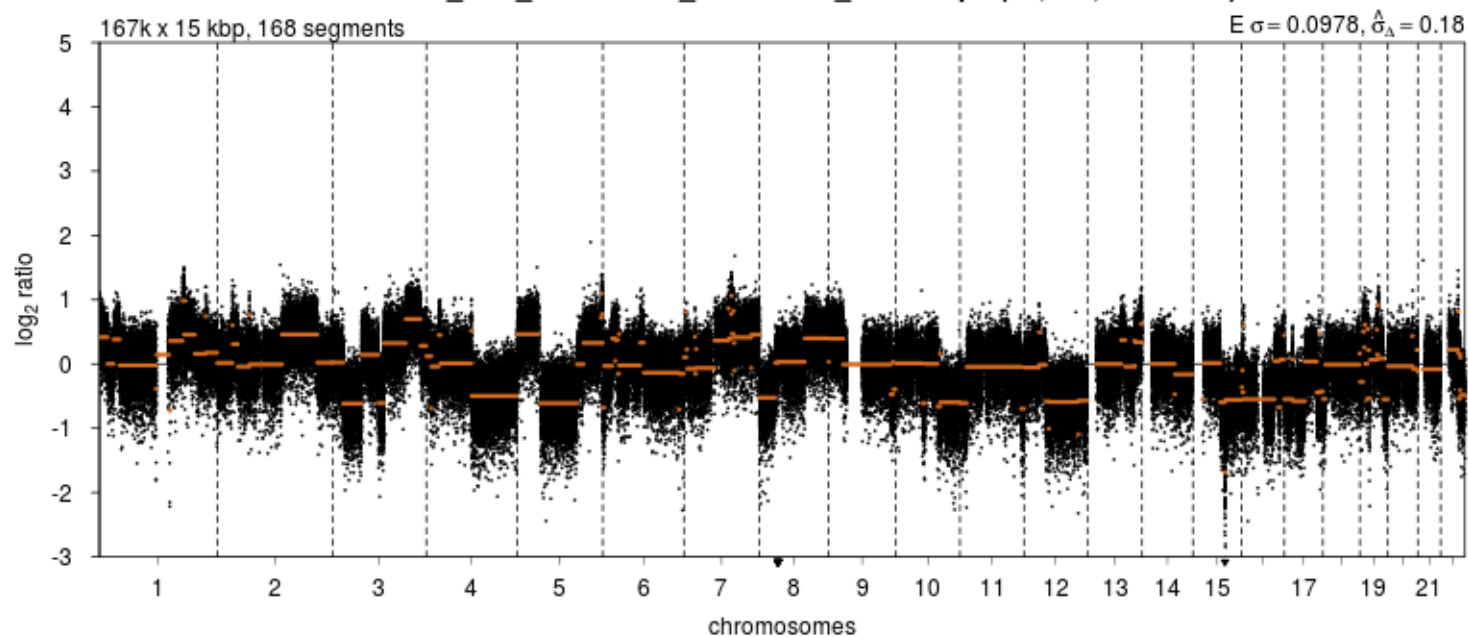
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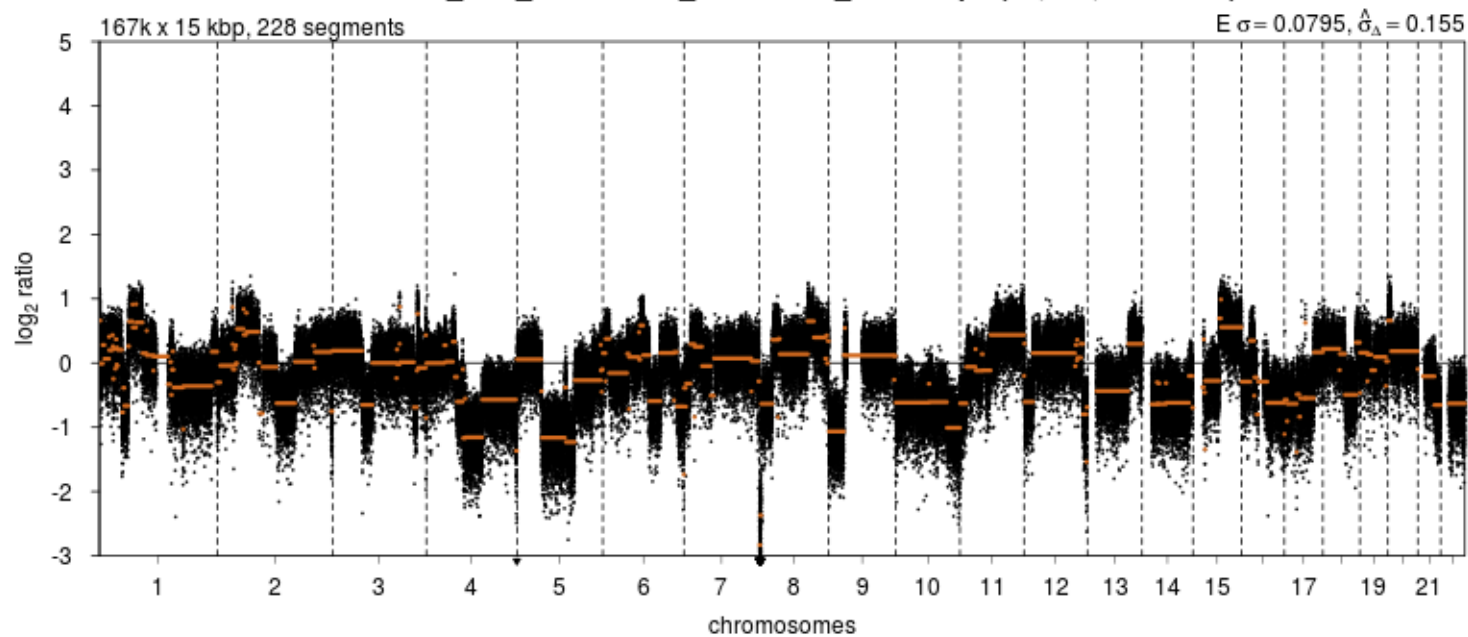
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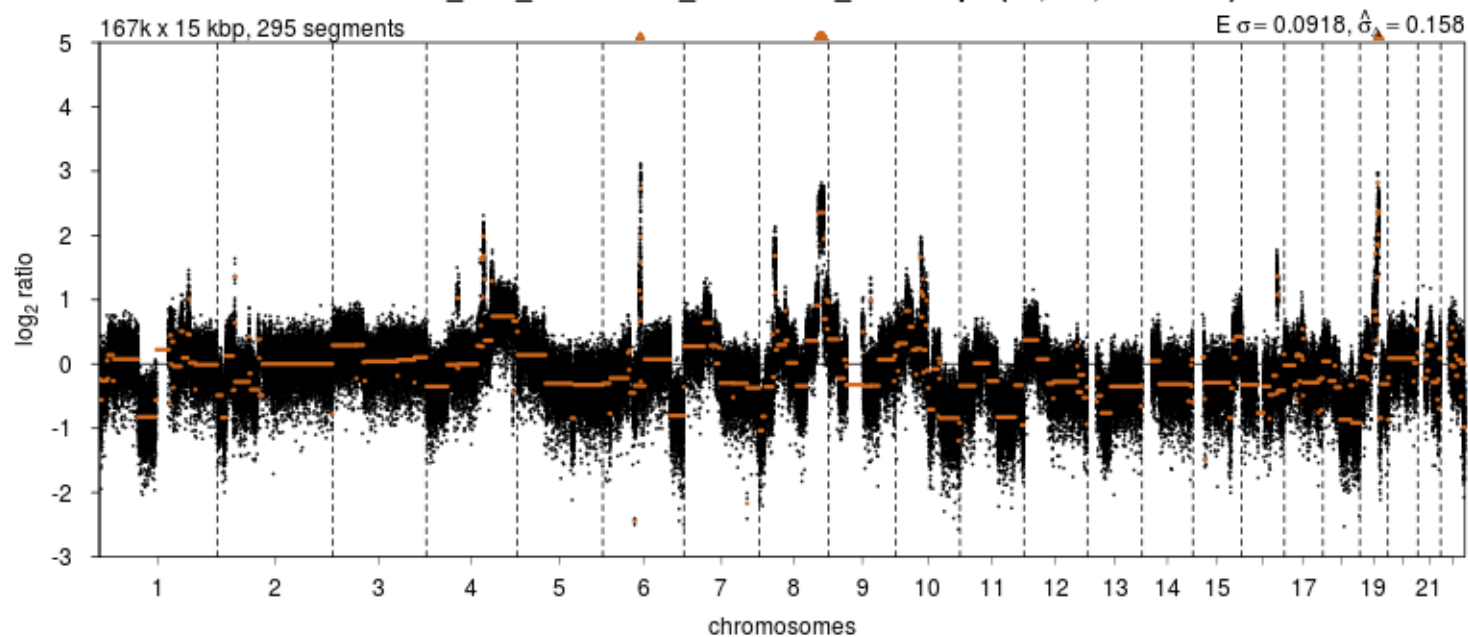
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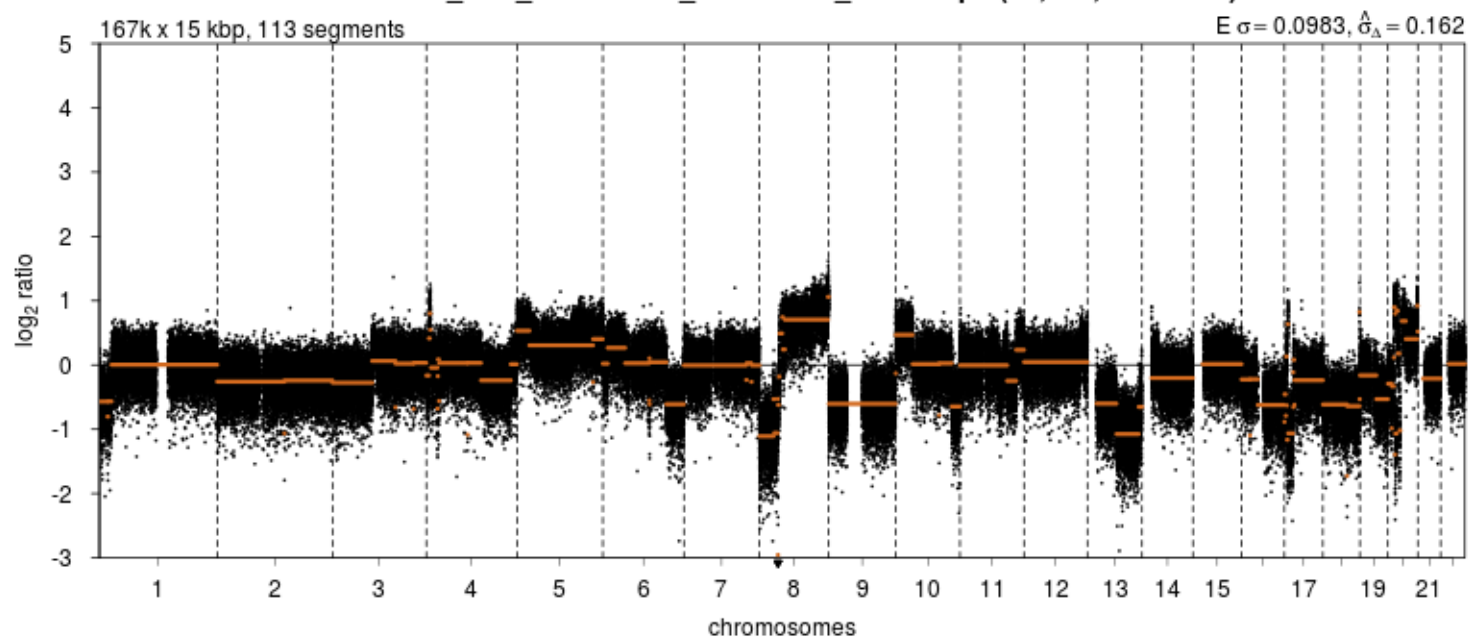
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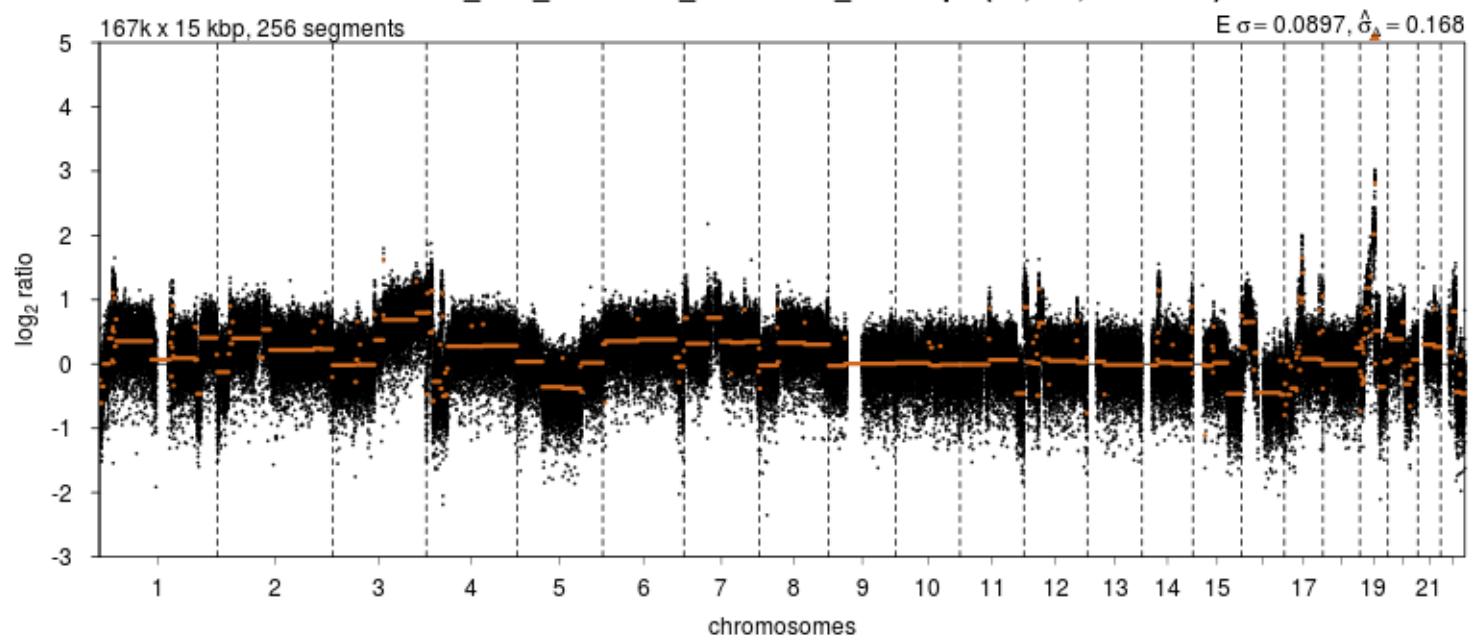
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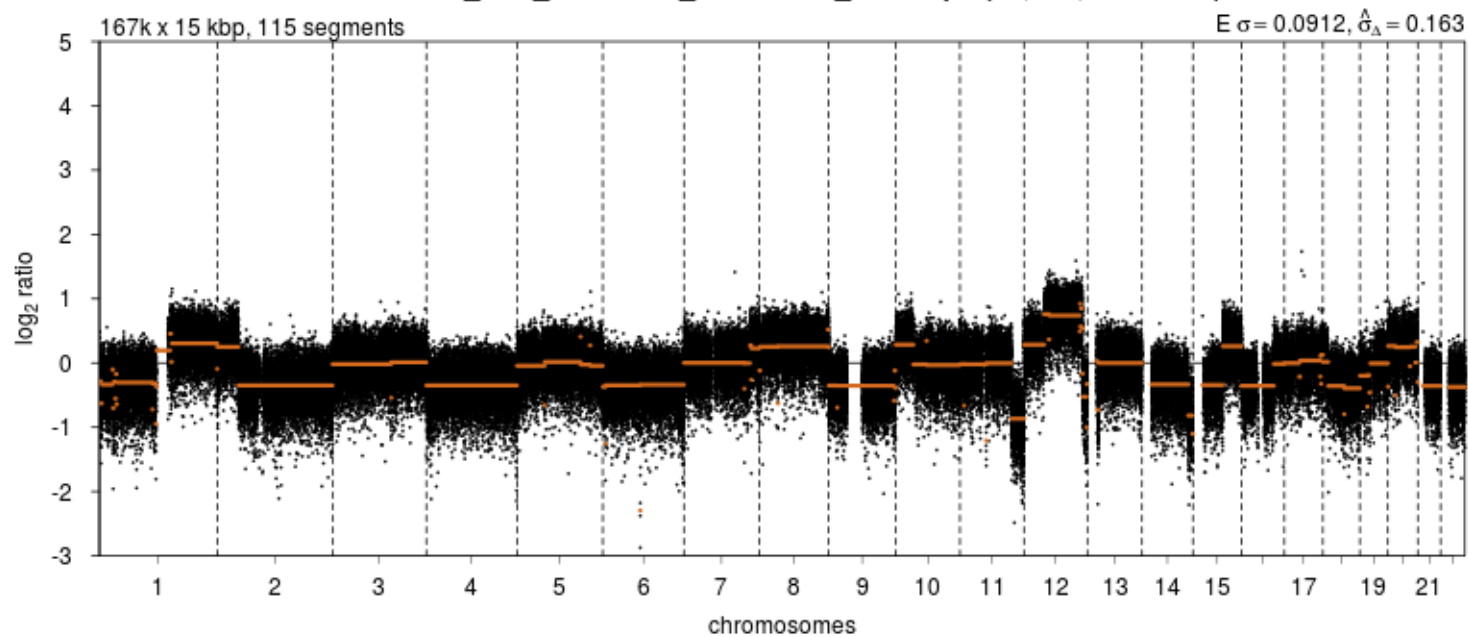
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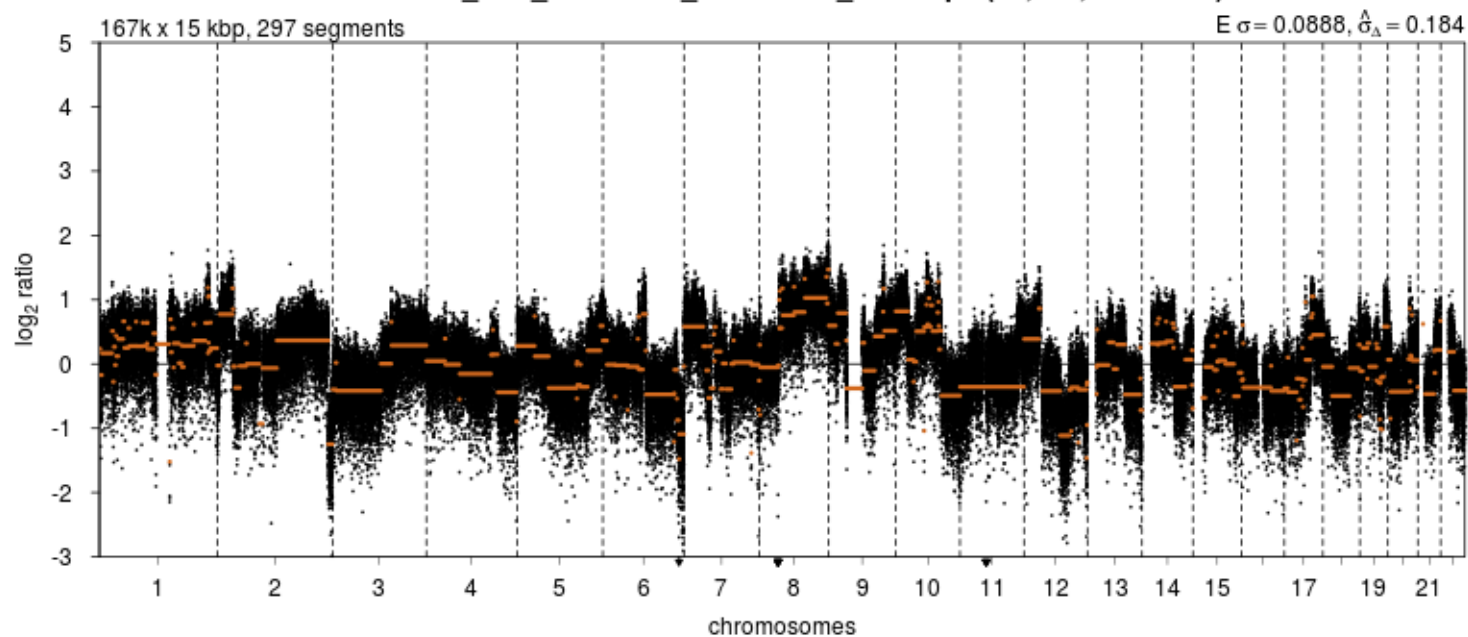
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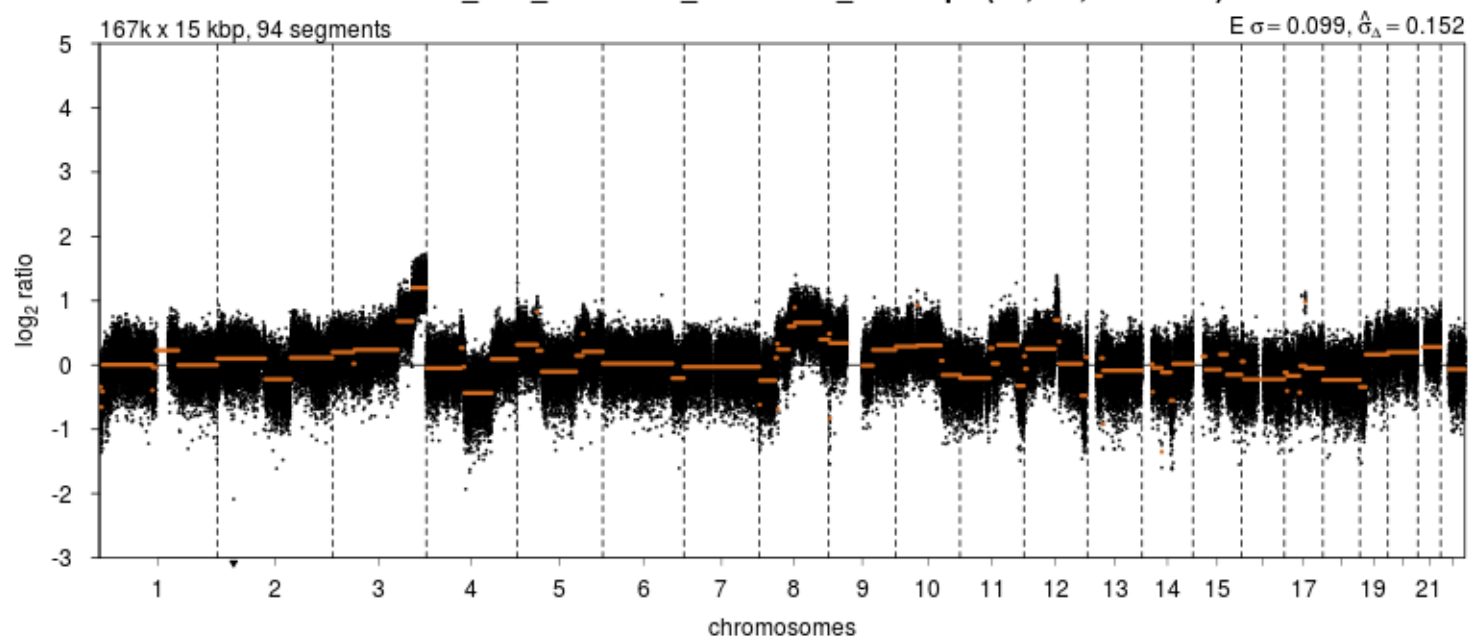
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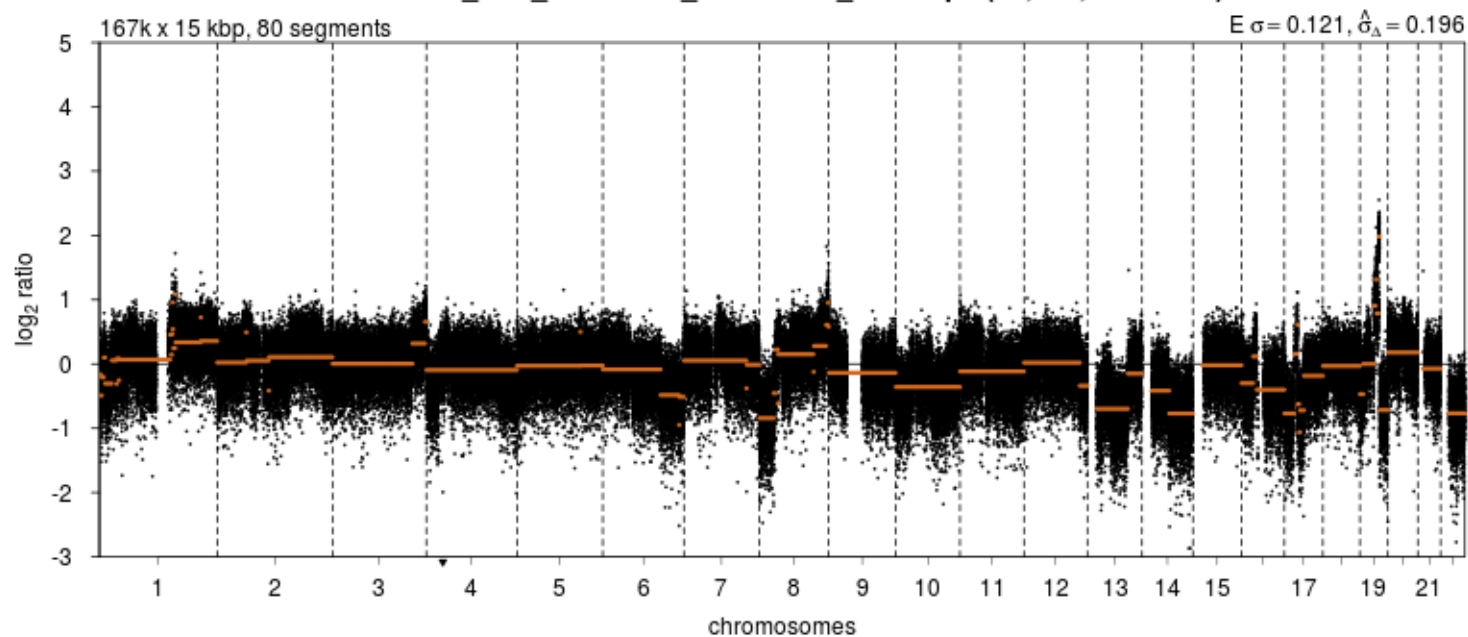
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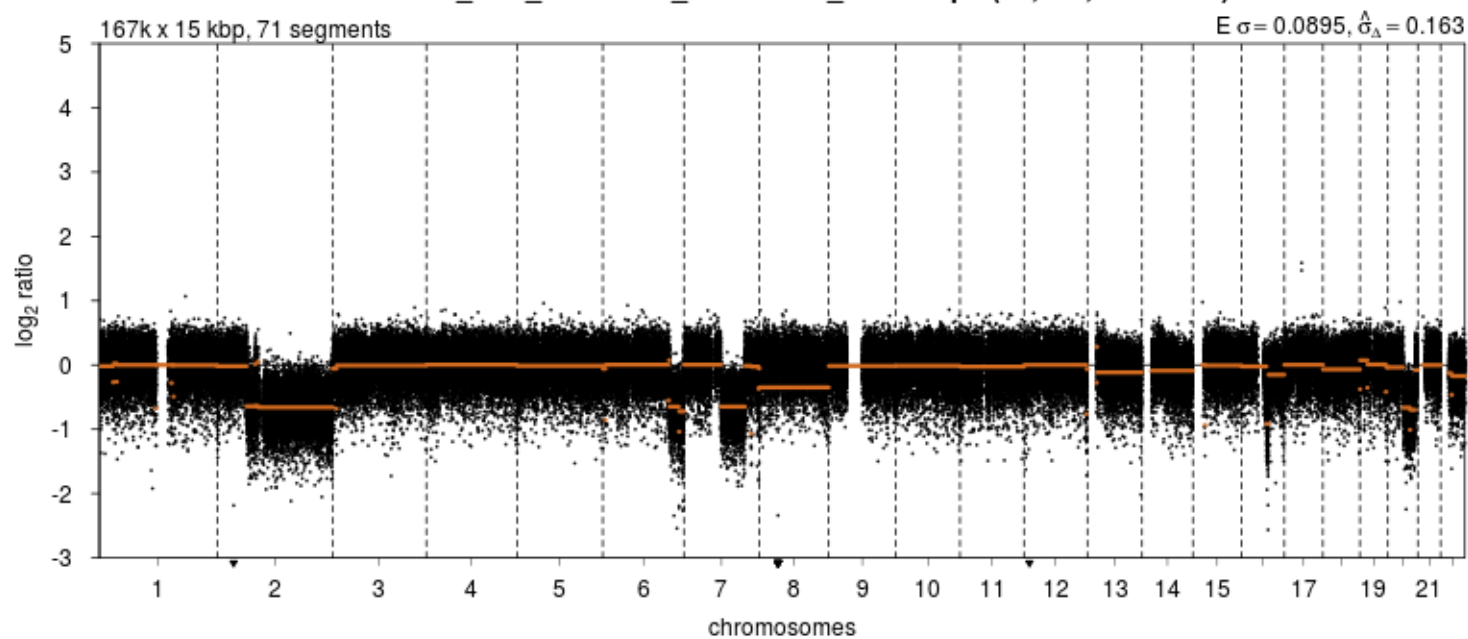
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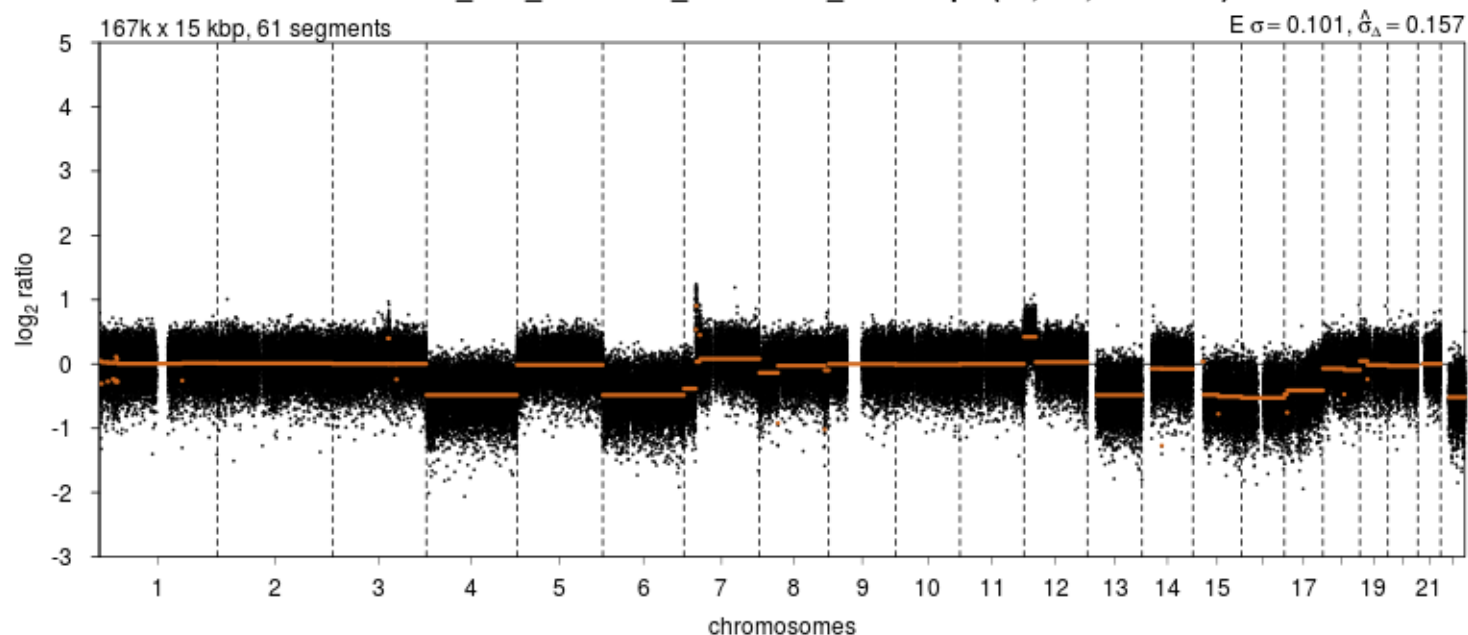
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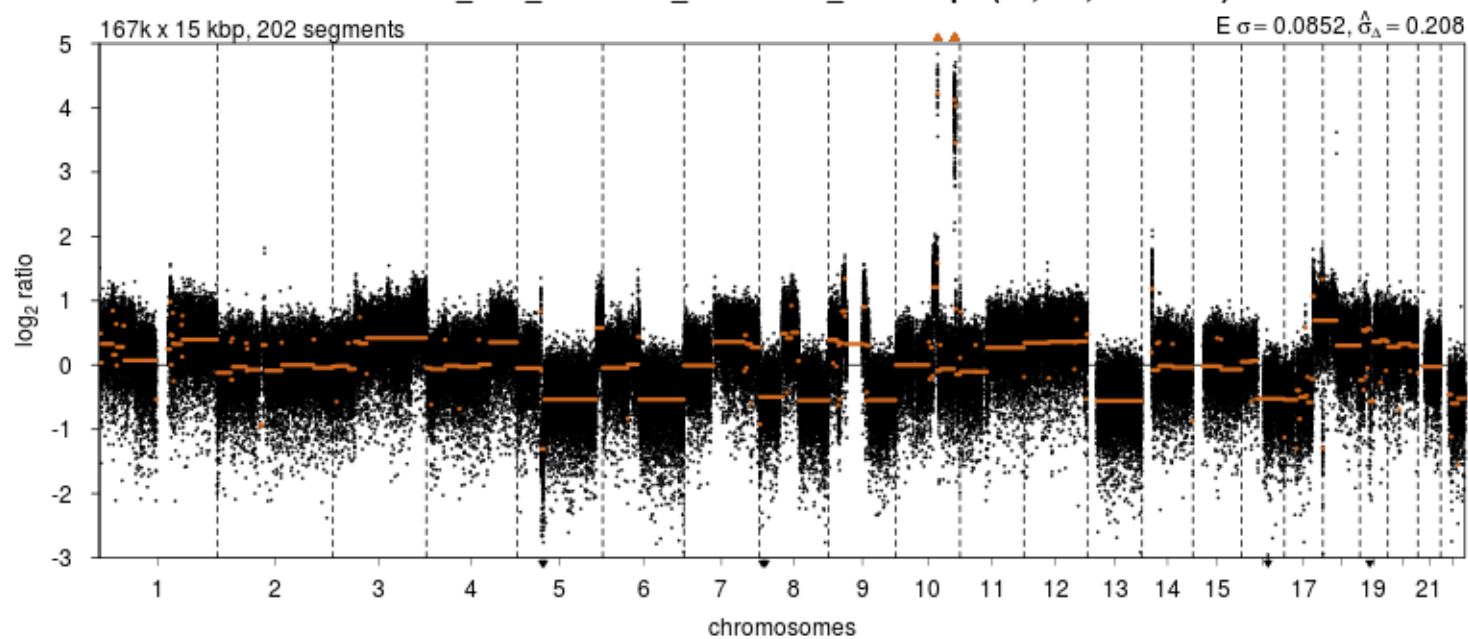
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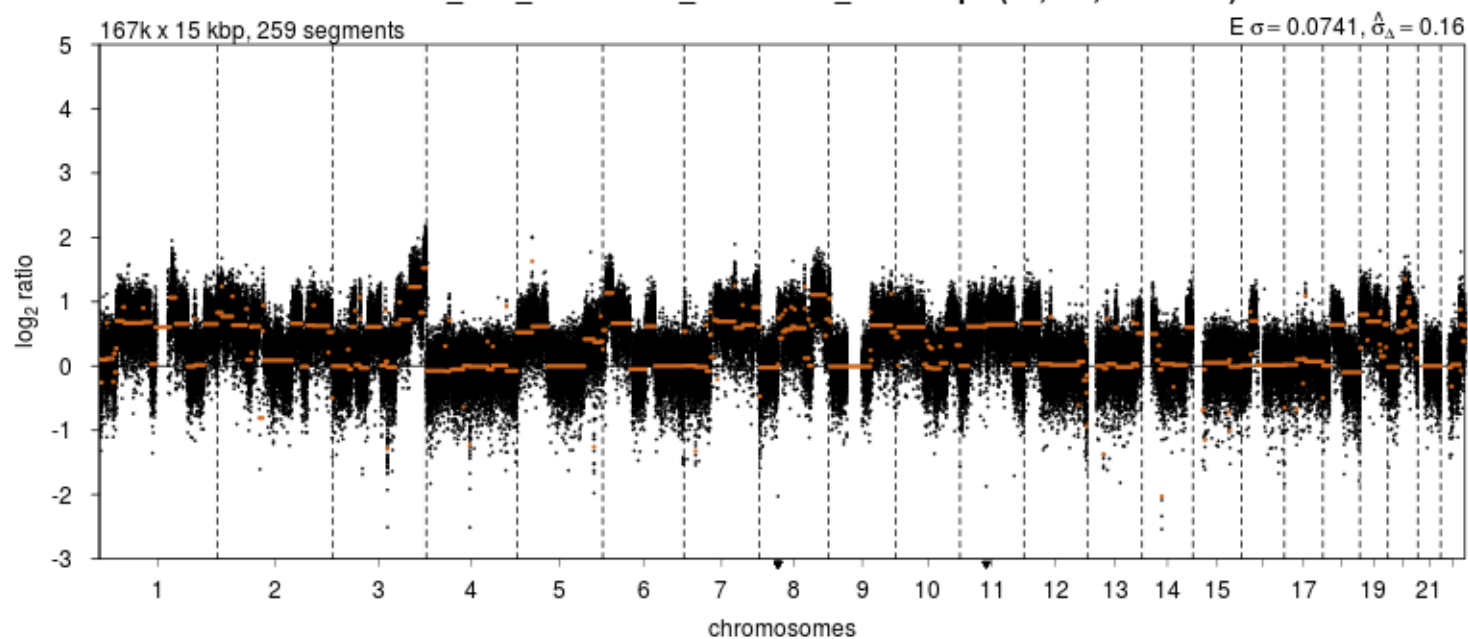
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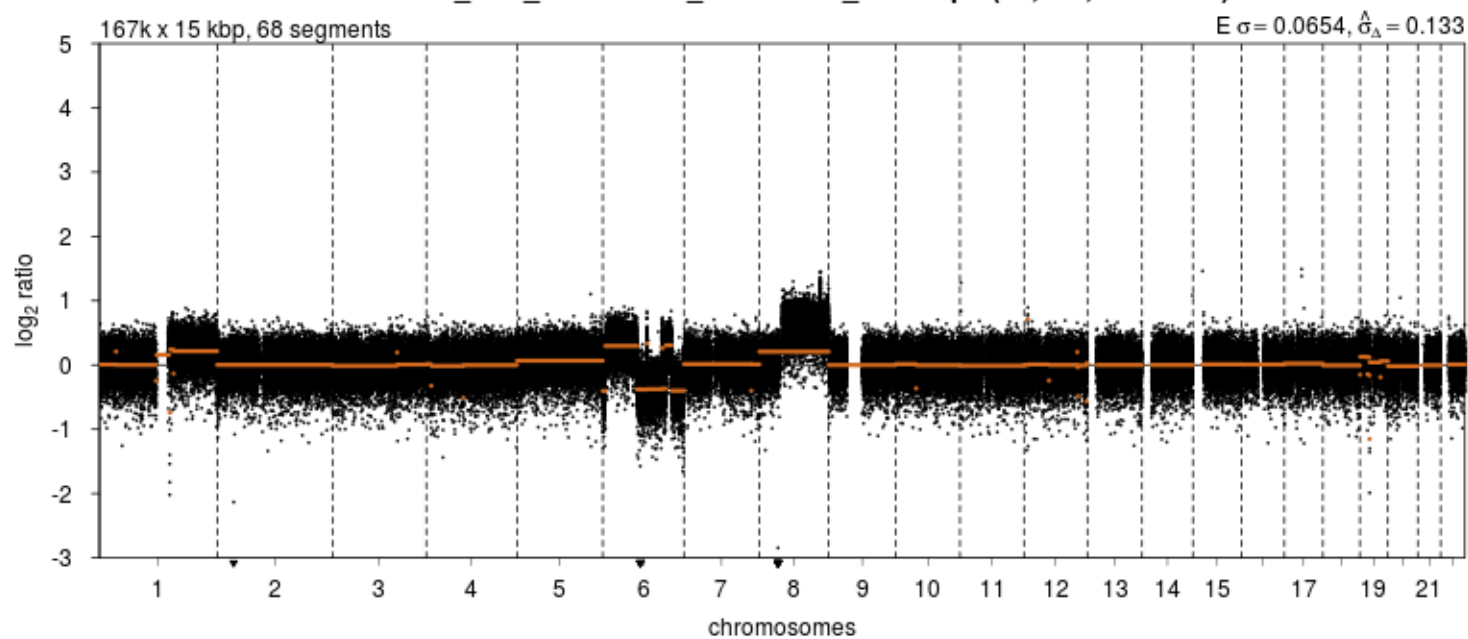
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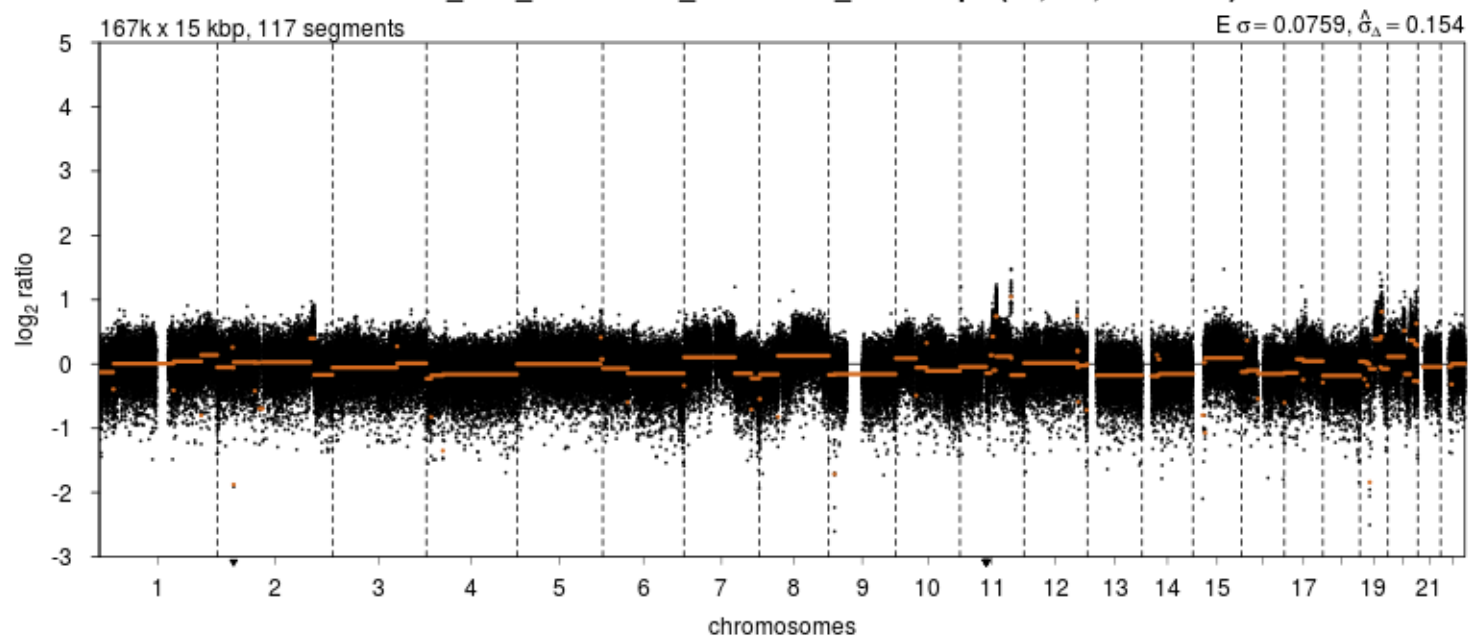
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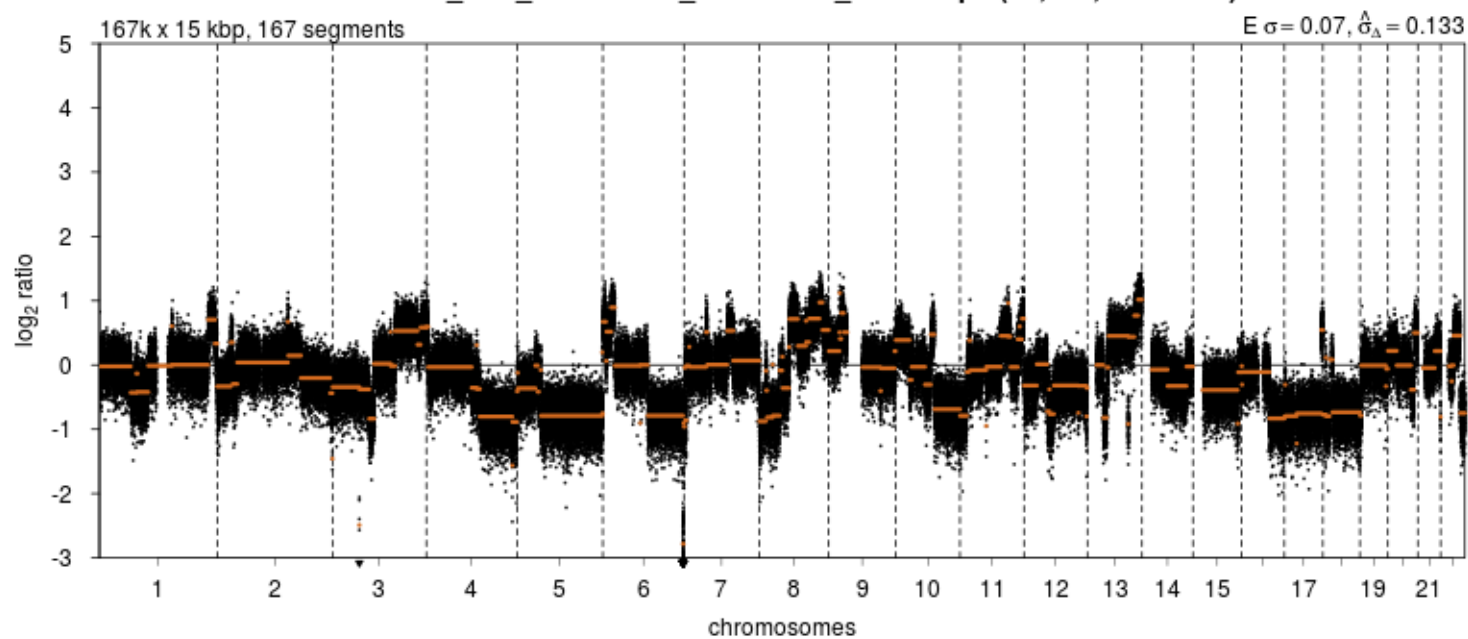
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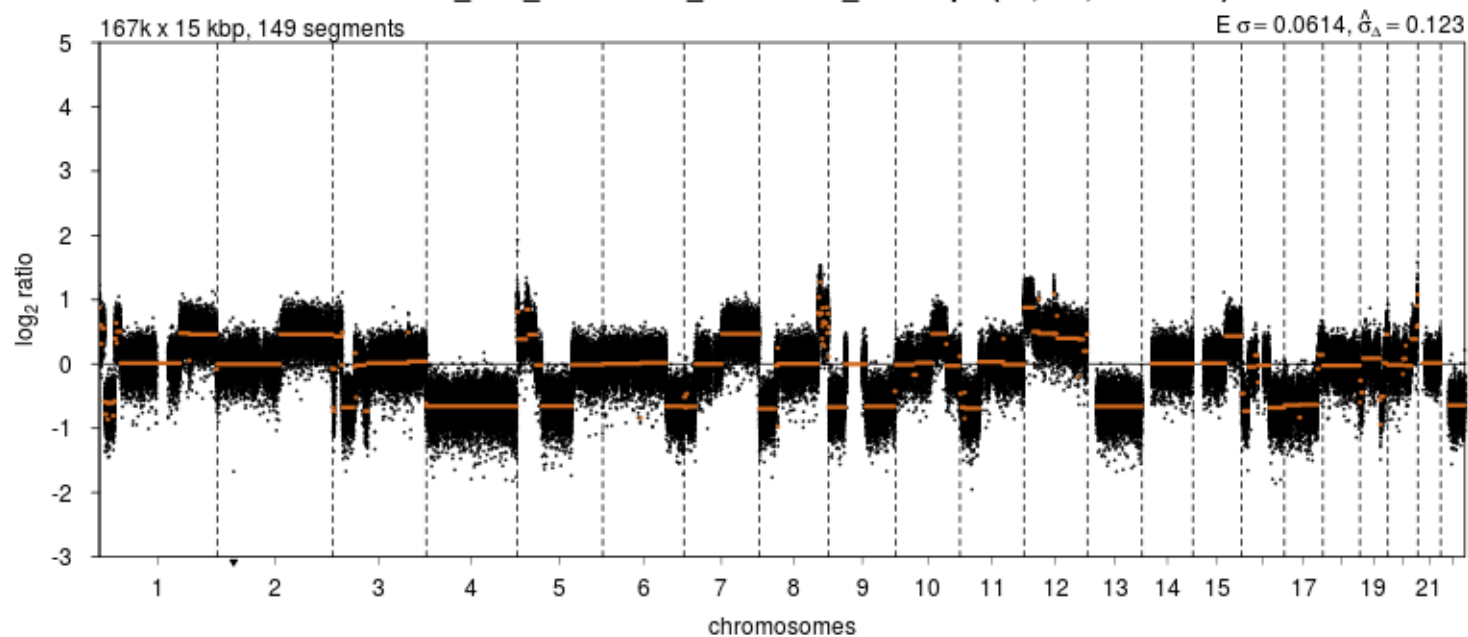
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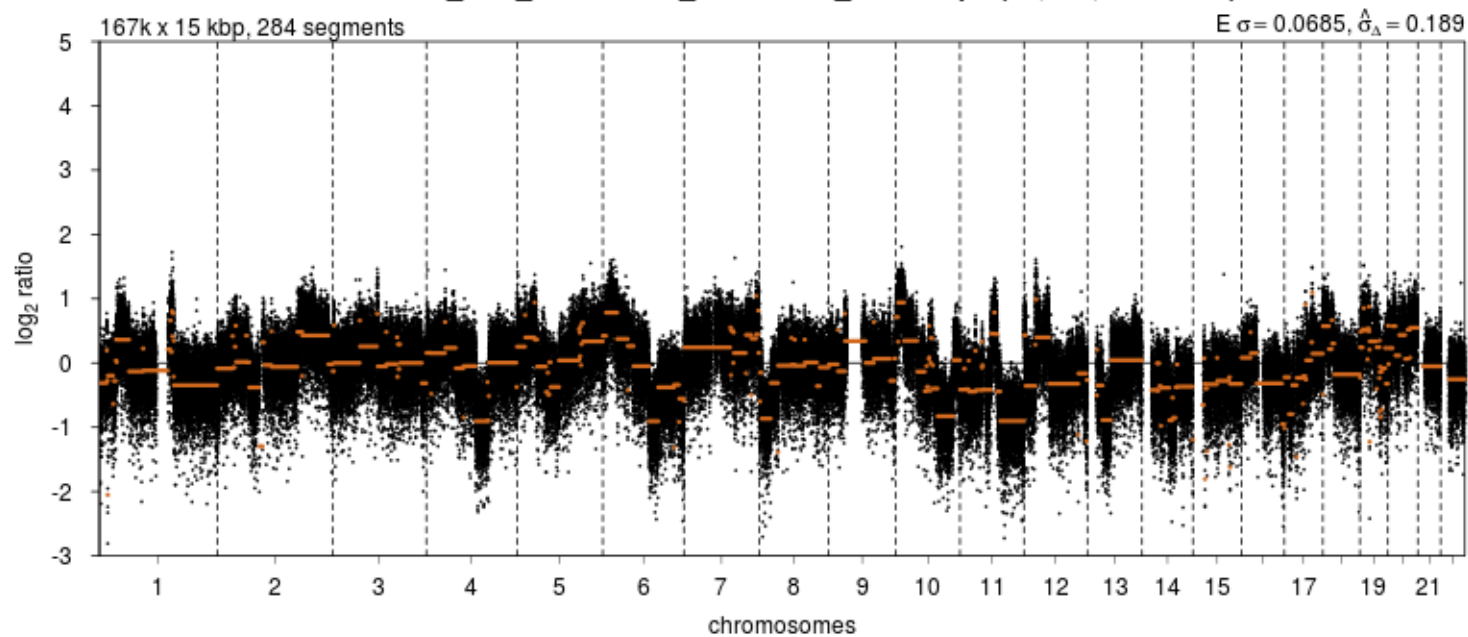
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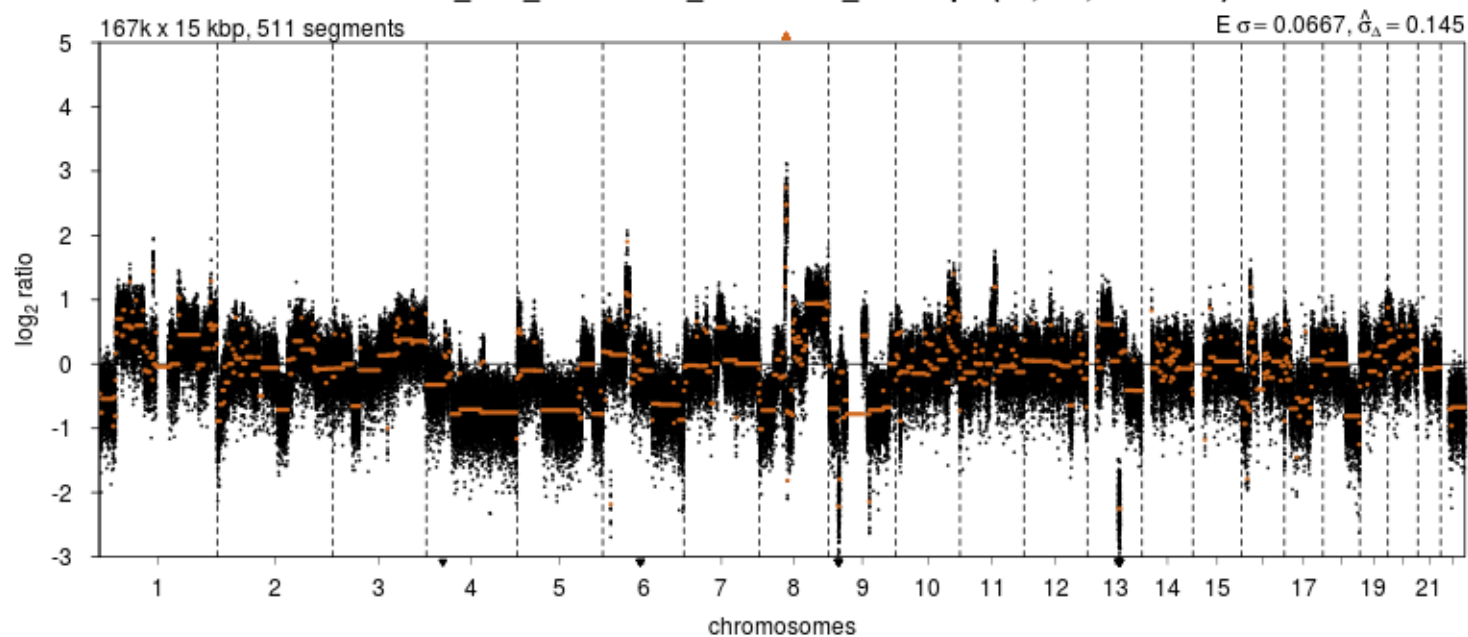
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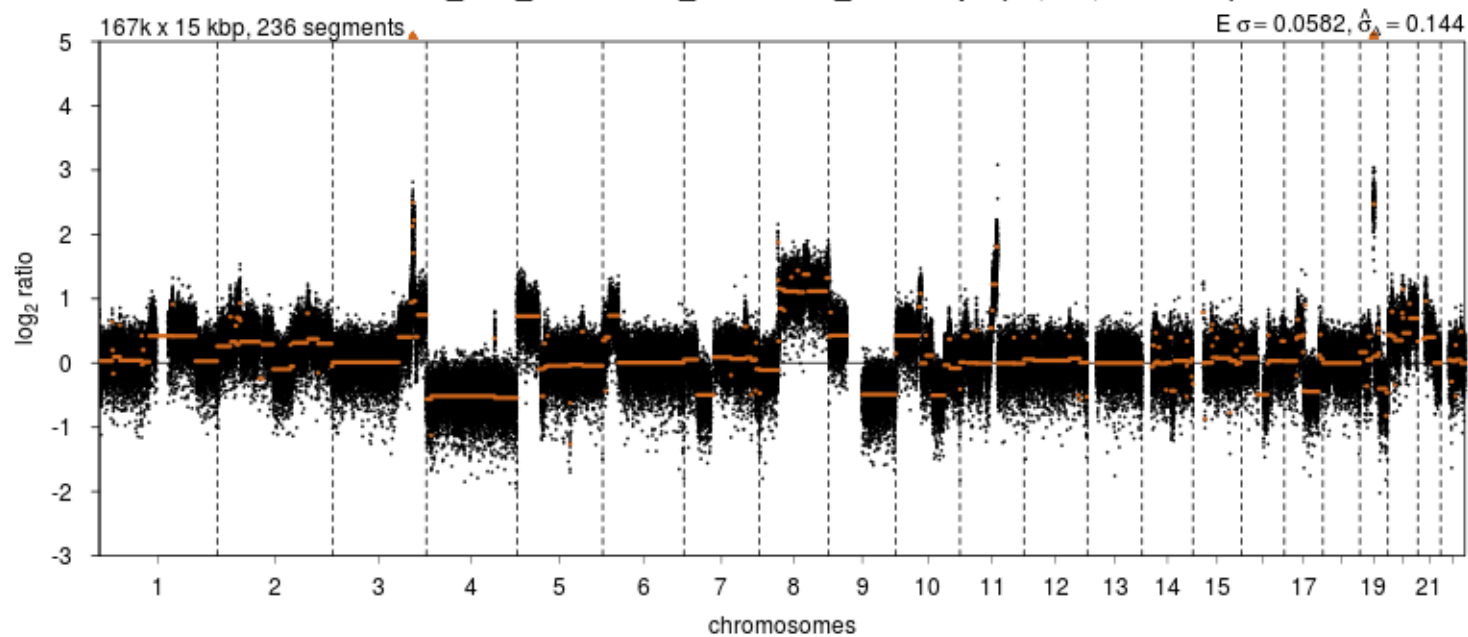
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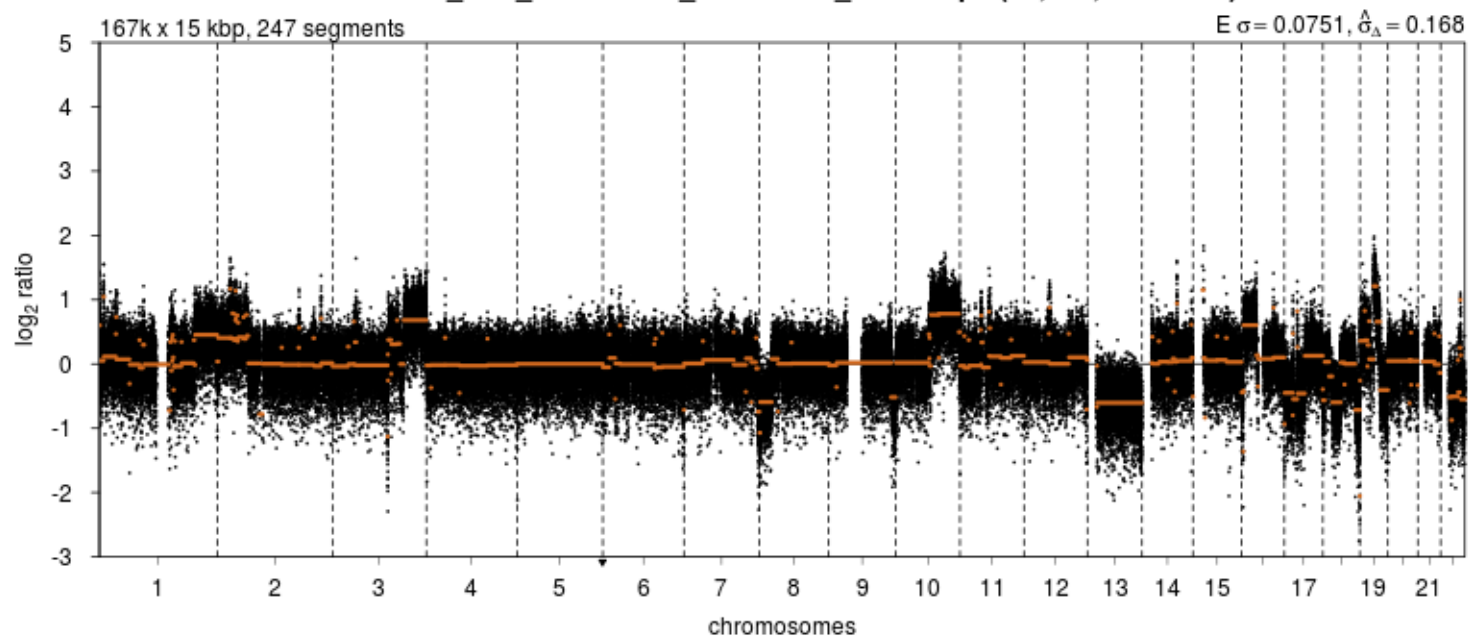
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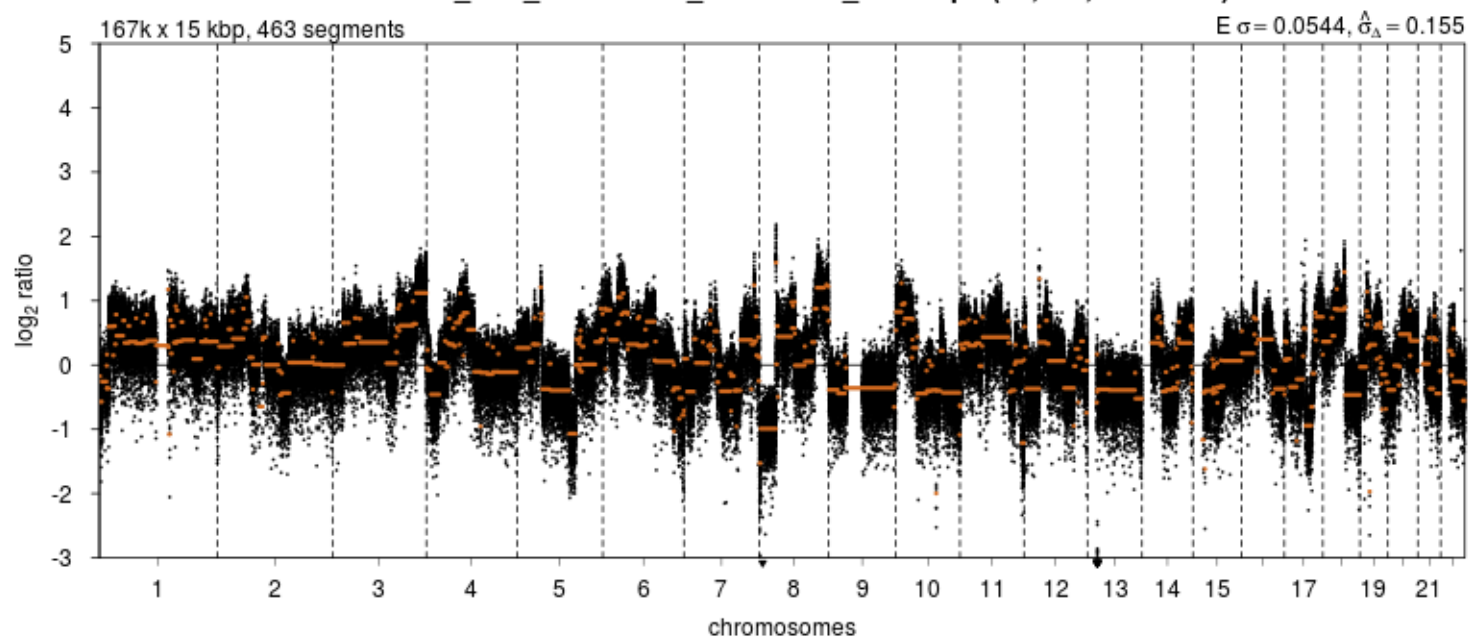
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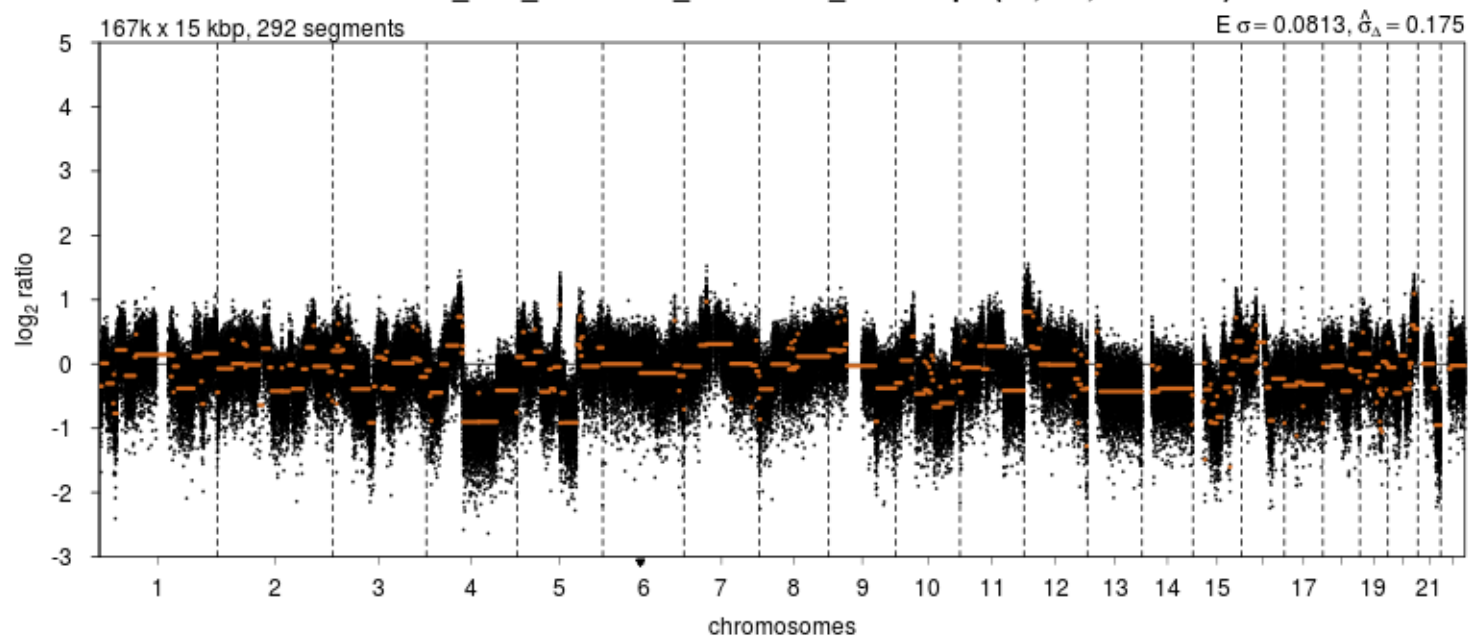
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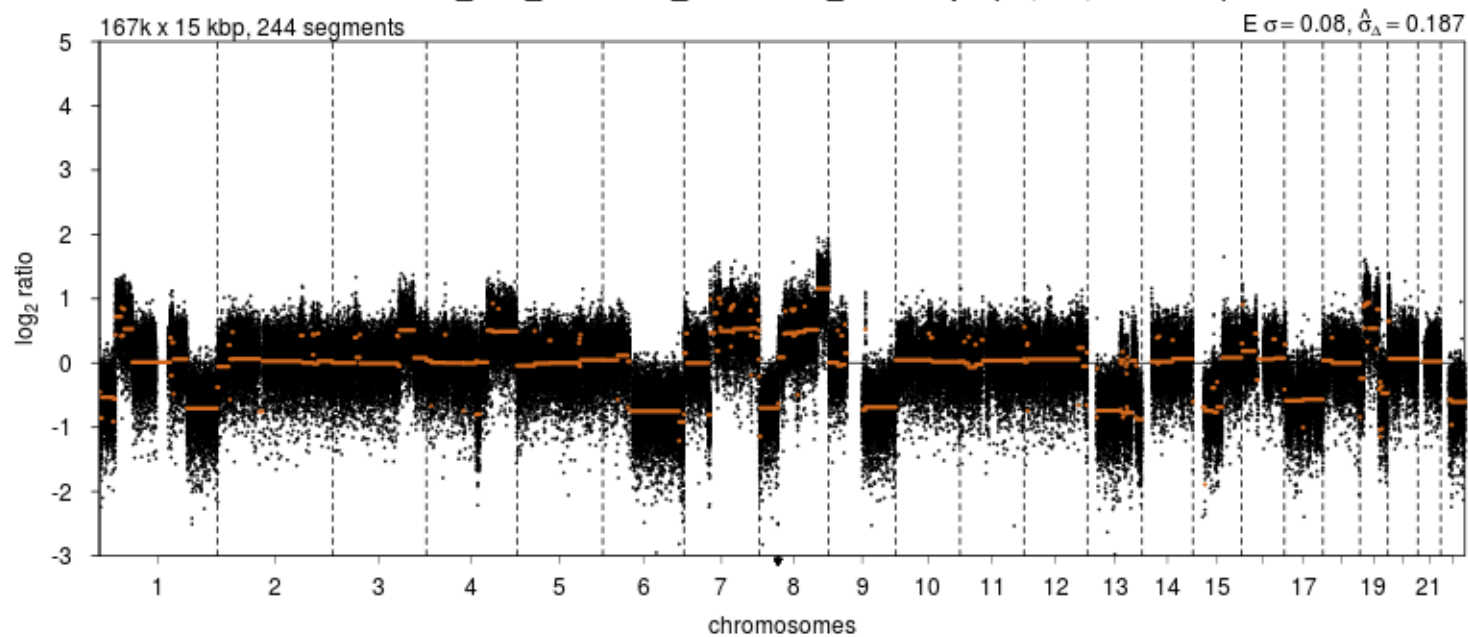
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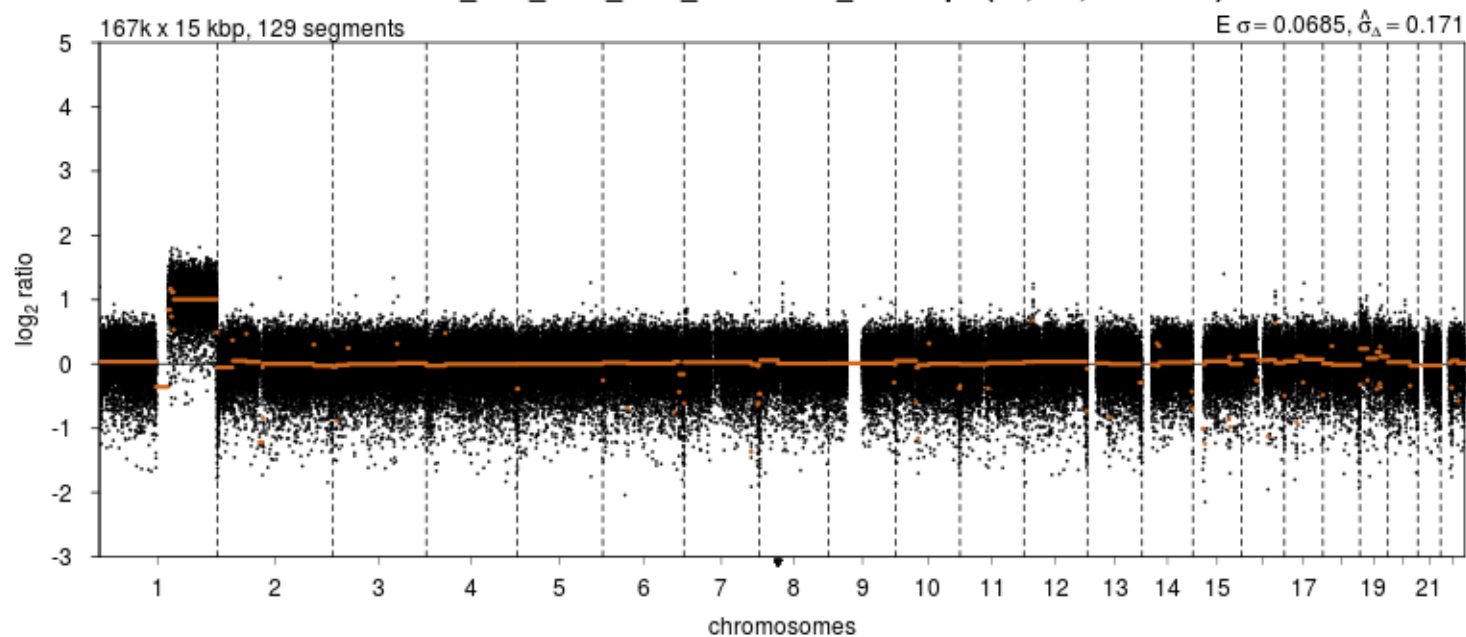
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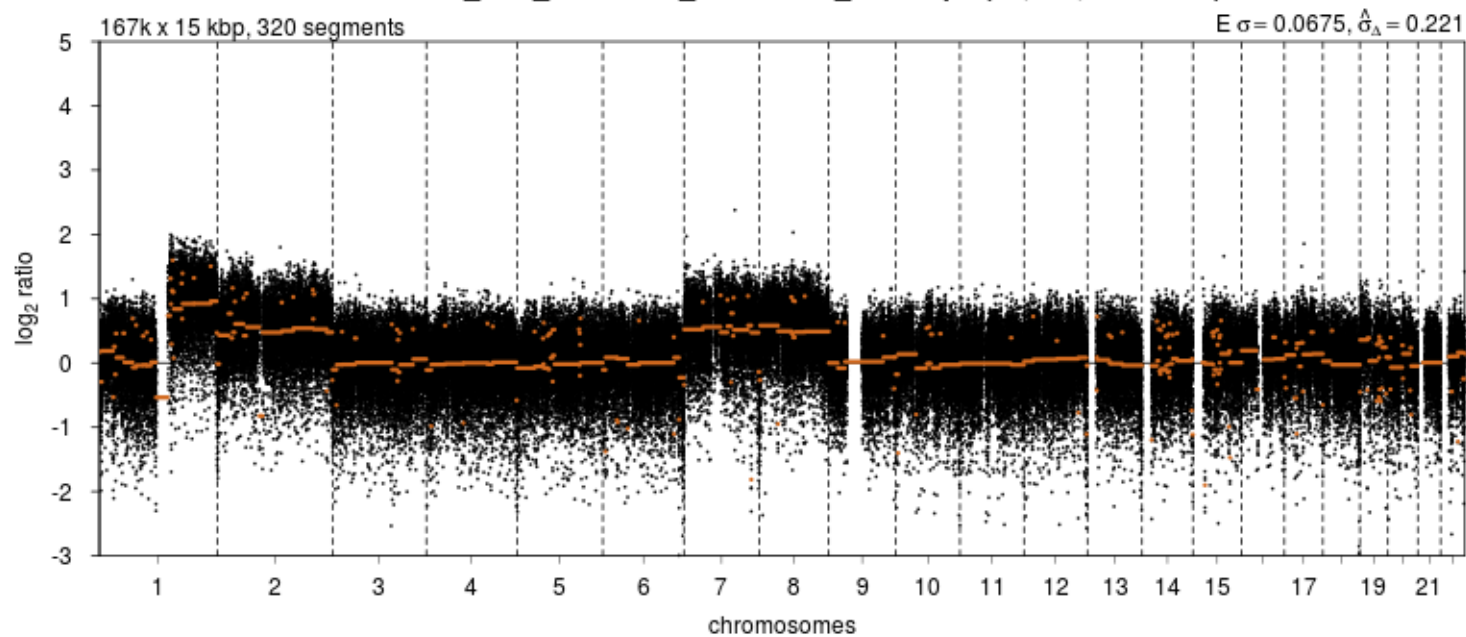
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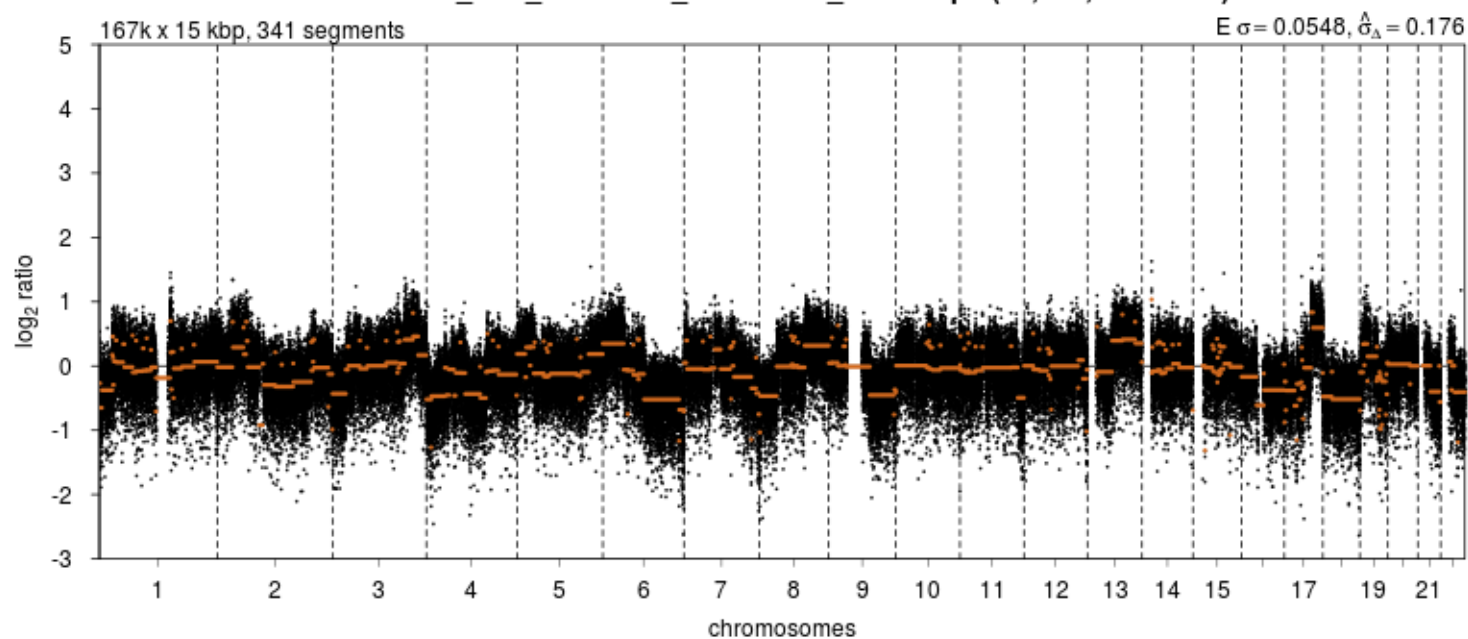
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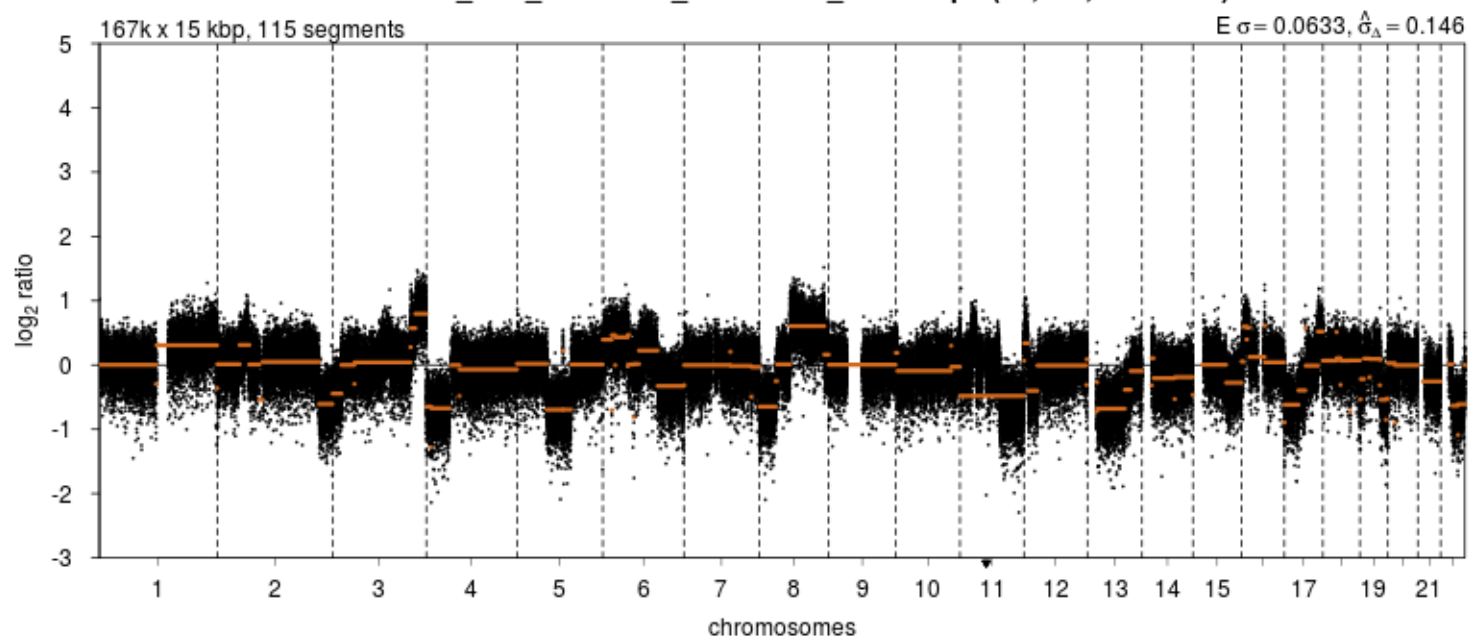
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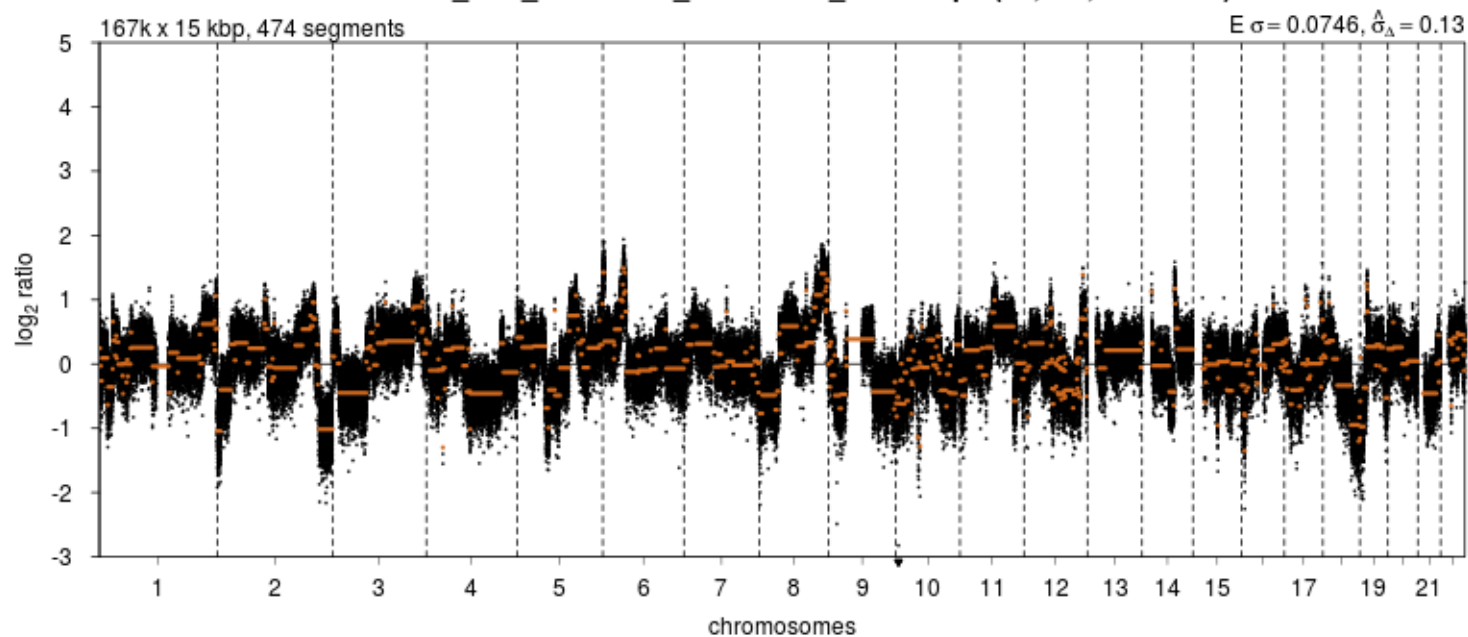
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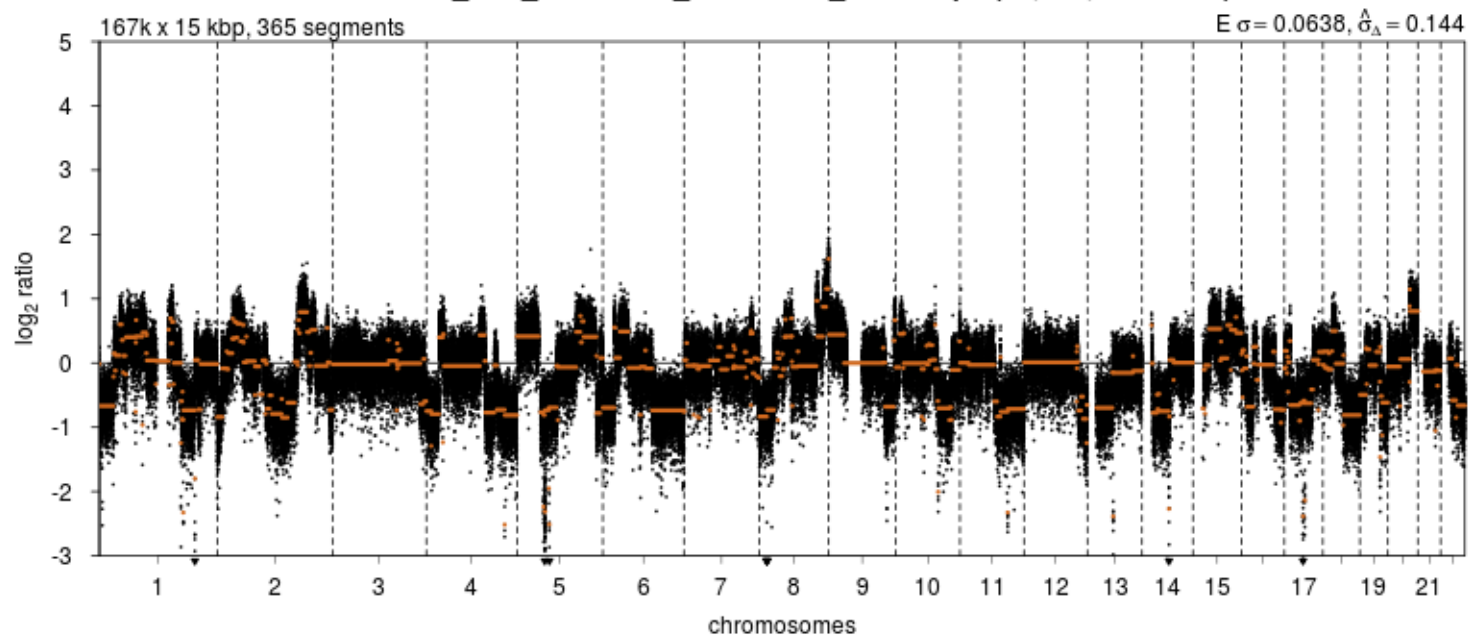
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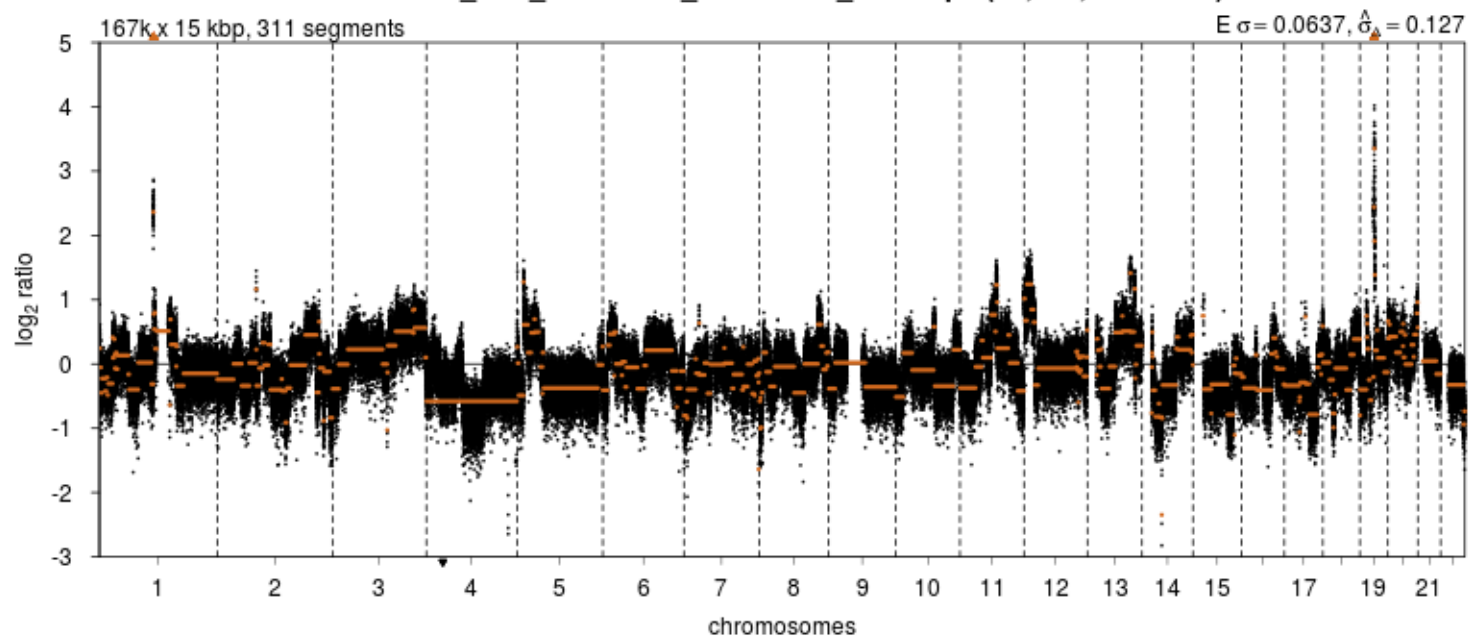
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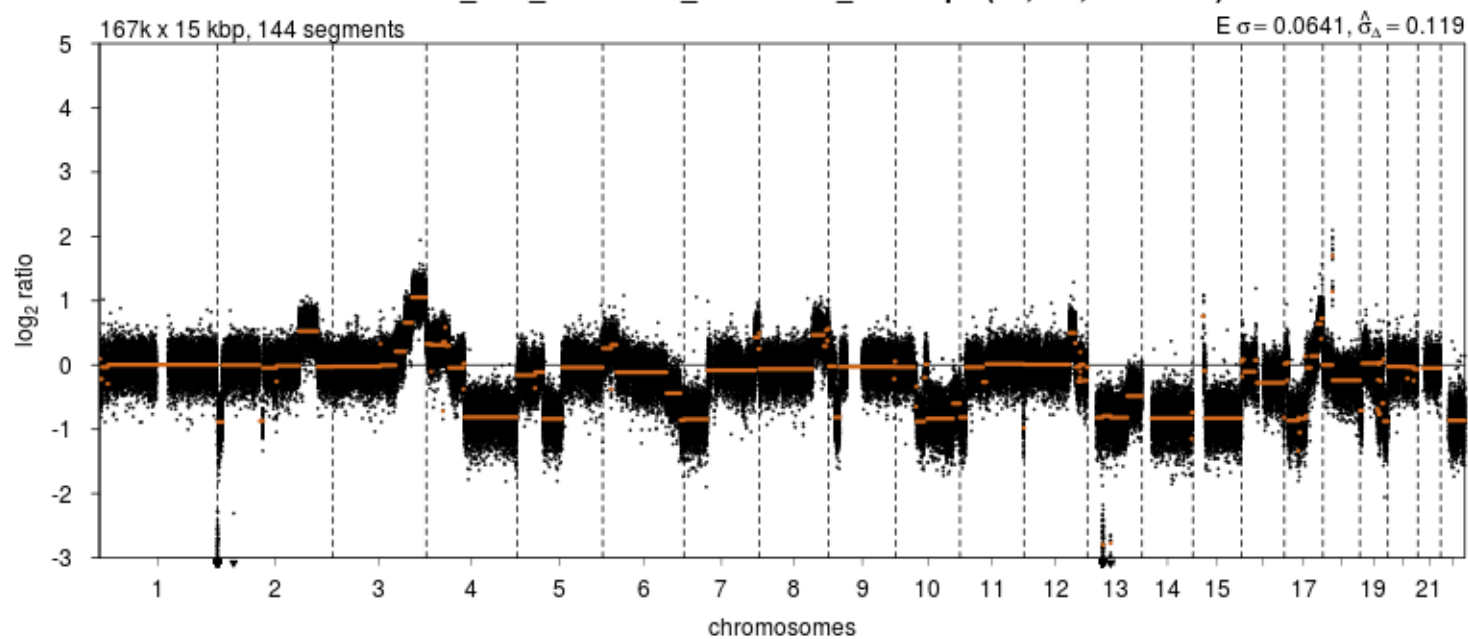
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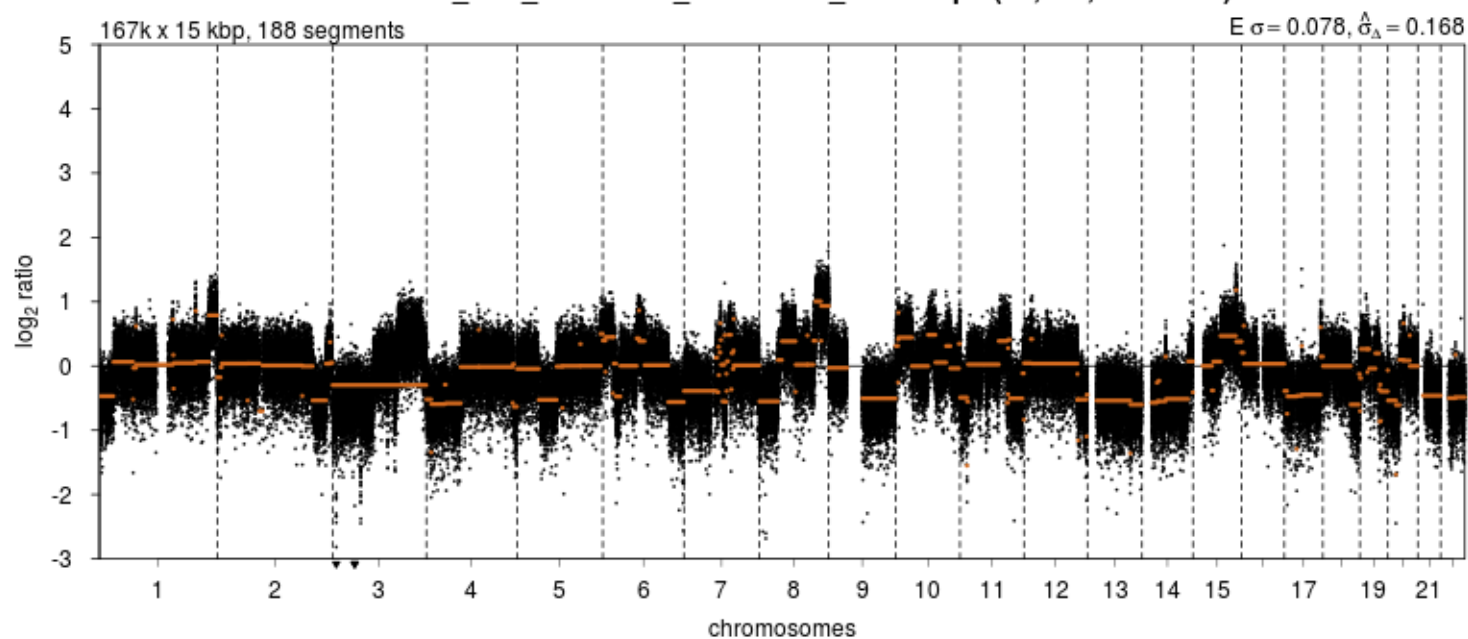
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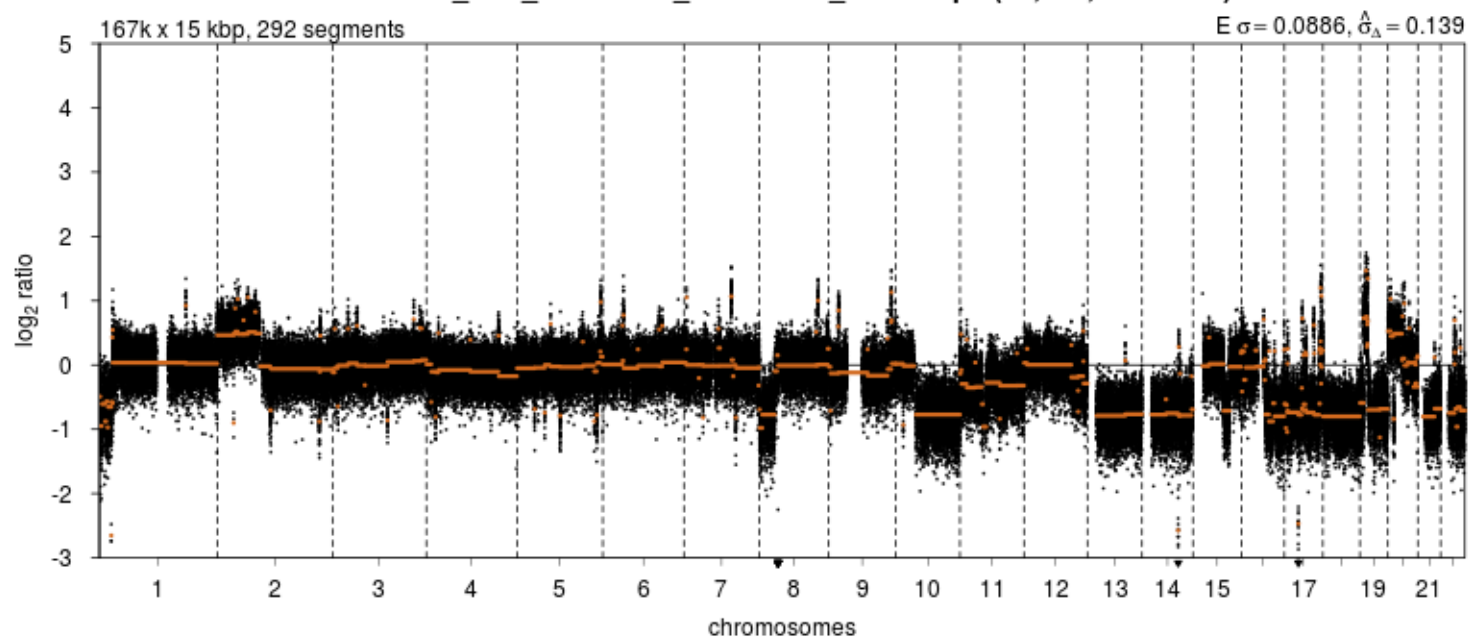
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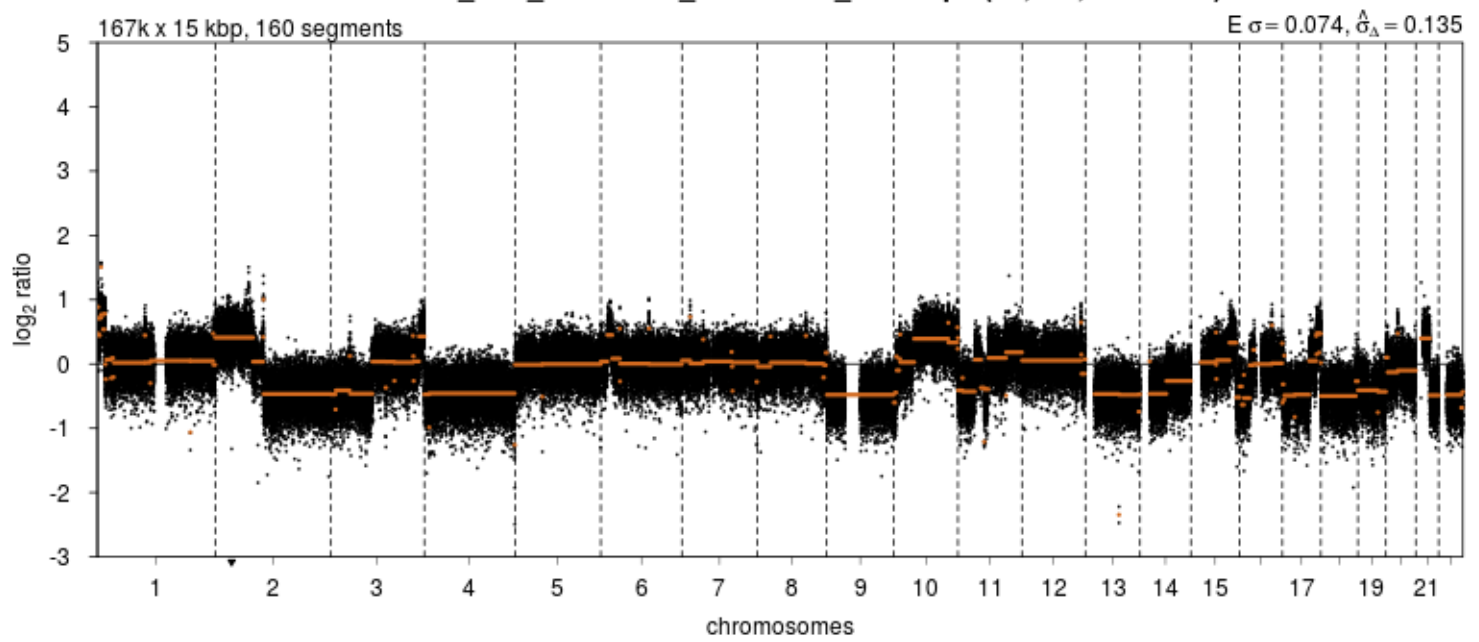
20170203_WW_0155-BWH_WW3600-2_S18.aln-pe (31,062,543 reads)



20170203_WW_0156-BWH_WW3600-2_S11.aln-pe (24,387,514 reads)



20170203_WW_0161-BWH_WW3600-2_S5.aln-pe (34,776,859 reads)



20170203_WW_0164-BWH_WW3600-2_S17.aln-pe (31,242,771 reads)

